SRI LANKA VETERINARY JOURNAL

VOL. 63 (2) July - December 2016

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LETTER TO THE EDITOR

ABSTRACTS

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Vector-borne diseases (VBD) which were mostly seen in the tropics, in locations with limited resources and surveillance (Gubler, 2009), have now reached high or medium occurrence in all continents, except Australia [CVBD (Companion Vector Borne Diseases) website].

A global re-emergence of VBDs, with a high frequency of transmission has been recorded due to multiple environmental and social factors, such as, increased human and animal bonding, mobility across borders, animal trade, global warming, resistance to pesticides, changes in agricultural practices, deforestation and reforestation and loss of biodiversity (WHO, Vector-borne diseases, February 2016). Pets are not considered a direct source of infection to people, but direct transmission from an infected animal can occur via accidental inoculation of contaminated blood or tissue.

This re-emergence of VBDs in humans and animals, has presented a 'One Health' issue highlighting the requirement for interaction between veterinary and human medical sectors for the benefit of animal and human health and the global environment (Braks et al., 2007; Day, 2011; Gubler, 2009). There are many challenges in achieving the above goals: (1) promoting detection of these diseases in animals and humans, (2) undertaking comparative and translational research (3) developing robust diagnostic tests and surveillance systems (4) studying the connection between pet animals and wildlife reservoirs (6) promoting awareness of pet owners on the importance of regular ectoparasite control, and (7) developing strategies to minimize the risk involved in pet animal mobility with regard to zoonotic diseases. This paper intends to highlight the complex clinical presentations associated with tick-borne diseases (TBD) based on scientific information and personal experiences on such diseases in dogs.

The organisms responsible for TBDs in South-East Asia and the Indian Subcontinent are: Anaplasma (phagocytophilum and platys), Babesia (vogeli, gibsoni and canis), Ehrlichia (canis, chaffeensis), Hepatozoon (Haemobartonella) and Rickettsia (felis, japonica, conorii, typhi & orientia tsutsugamushi) (CVBD). Vector-borne hemoparasites in dogs in Sri Lanka are Anaplasma platys, Babesia gibsoni, Babesia canis, Ehrlichia canis and Hepatozoon canis. Presence of Rickettsial parasites (R. conorii, R. typhi, Orientia tsutsugamushi) also had been detected serologically (Nanayakkara et al., 2013).

Furthermore, intermittent, persistent infections or co-infections caused by Babesia, Anaplasma, Ehrlichia, Hepatozoon, Rickettsia and Mycoplasma haemocanis (Haemobartonella) are commonly encountered by practitioners. Co-infections of Ehrlichia, Babesia and Hepatozoon can occur particularly in endemic areas since the same tick species can transmit several pathogens. Such infections probably explain the variations in clinical presentation, pathogenicity and response to therapy (Shaw et al., 2001).

Babesia organisms are broadly divided by their size into small and large. The large babesia which were previously known as Babesia canis are reclassified as B. canis, B. vogeli, B. rossi and Babesia sp (Cocco). The small Babesia previously known as Babesia gibboni are reclassified as B. gibboni and B. conoradi; Babesia microti as Theileria annae; and Babesia equi as Theileria equi (Irwin, 2009). Babesiosis in cats is less common and is manifested as an afebrile, chronic, low-grade disease, with anorexia, lethargy, anemia, depression, and occasionally icterus. Feline Babesiosis is caused by Babesia felis, Babesia catti (India), B. canis (Europe), B. canis presentii (Israel), B. vogeli (Thailand).

Ehrlichia and Anaplasma spp. are closely related with four distinct clades: Anaplasma, Ehrlichia, Wolbachia and Neoicketttsia gene groups (Dumler et al., 2001; Ferla et al., 2013).

Ehrlichia gene group- Ehrlichia canis, E. chaffeensis and E. Ewingii

Anaplasma gene groups- E. Phagocytophilum (formerly known as E. equi) and E. platys (synonym Anaplasma platys) Ehrlichia Phagocytophilum (Anaplasma) infection in neutrophils has become increasingly significant in human, canine and feline populations causing granulocytic ehrlichiosis. Neorickettsia gene groups - E. sennetii, E. risticii and Neorickettsia helminthoeca

Ehrlichia chaffeensis cause human monocytic ehrlichiosis, Ehrlichia ewingii causes human granulocytic ehrlichiosis, and Anaplasma phagocytophilum cause human anaplasmosis (formerly known as human granulocytic ehrlichiosis, or HGE).

Transmission

The transmission of TBD pathogens can occur in different ways. In biological transmission, the pathogen

http://doi.org/10.4038/slvj.v63i2.9
Tick-borne diseases undergo some biological development in the body of the arthropod vector in order to complete its life cycle. Mechanical transmission is a simple transfer of the organism on contaminated mouth parts or other body parts, without multiplication or developmental change of the pathogen in the arthropod (Gubler, 2009). In addition to biological and mechanical transmission, direct transmission of TBD can occur through dog bites, contaminated equipment, transplacentally and via blood transfusions. Although *Ehrlichia canis* and *Hepatozoon canis* are transmitted by ticks, *E. canis* is transmitted through the saliva of ticks during a blood meal, while *H. canis* is transmitted by ingestion of a tick containing mature sporozoites (Baneth et al., 2007; Harrus and Waner, 2011).

Hemotrophic mycoplasmas (hemoplasmas), formerly classified as Haemobartonella and *Eperythrozoon* species, with a single circular chromosome are transmitted by *Rhipicephalus sanguineus* and *Dermacentor reticulatus* ticks, from a blood transfusion and also directly through saliva during a fight (Willi et al., 2006; Nascimento et al., 2012). They adhere to the surface of the erythrocytes and cause extravascular hemolysis. *Mycoplasma hemofelis, M. hemocanis* and *Candidatus* are closely related (Sykes and Tasker 2016; Braga et al., 2012). Hemoplasmas in dogs are *M. haemocanis, Candidatus M. haematoparum* (Nascimento et al., 2012). *Mycoplasma haemofelis, Candidatus Mycoplasma haemonunium, Candidatus Mycoplasma turicensis* cause feline infectious anemia in the presence of retrovirus-induced immunosuppression or interaction with blood progenitor cells.

**Phagolysosome**

Extrinsic incubation period (time required to develop inside the arthropod and to progress to the infective stage) is generally 7 to 14 days (Gubler, 2009). Mitochondria are important for the division of Babesia by binary fission (10 h *in vitro*) which depends on nucleic acid synthesis and energy metabolism via aerobic and anaerobic glycolysis, converting glucose to lactate (Rajapakshage et al., 2012). The mitochondrial DNA (mtDNA) consists of three functional genes essential for energy metabolism.

- cytochrome c oxidase subunit I (COXI) - electron transfer in respiratory chain
- cytochrome c oxidase subunit III (COXIII) - assembly and stabilization of the entire cytochrome complex
- cytochrome b (CYTb) - subunit of COXIII - electron transfer

*Ehrlichia* organisms survive and multiply in the infected cell by their ability to inhibit the fusion of the phagosomeslysosome (Park and Rikihisa, 1991; Wells and Rikihisa, 1988).

**Immunity**

The cell-mediated immunity (CMI), the primary but not exclusive, is critical for the outcome of an infection (Waner et al., 2001). Macrophages play a leading role in CMI by phagocytosing and killing of organisms by O₂-dependent and O₂-independent mechanisms, and in initiating an acute phase response by releasing cytokine leading to a humoral immune response.

Cytokines contribute to both host pathology and host immunity (Hemmer et al., 2000). The T-cell-induced immunity and proinflammatory cytokine, Interferone gamma (IFNγ) secretion are predominant mechanisms for recovery from and immunity to ehrlichial infections (Waner et al., 2001). The cytokine IFNγ released from Th1 cells, macrophages and natural killer (NK) cells, enhance Tumor necrosis factor alpha (TNFα) expression which up-regulate the expression of adhesion molecules in endothelia, promoting sequestration of organisms to endothelia (Hemmer et al., 2000; Irwin, 2009; Tajima and Rikihisa, 2005). Furthermore, the recruit of leukocytes by TNFα lead to changes in vascular epithelium resulting in increased vascular permeability, oedema and tissue injury. Therefore, TNFα production is detrimental to the host as it enhances inflammation, leading to abnormal perfusion, tissue hypoxia, sepsis and systemic inflammatory response (SIRS). Increased expression of cytokines, IL-10 and IL-4 is important for resolution of cause extravascular hemolysis.

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neorickettsiosis infections can be distinguished by western immunoblotting (Harrus and Waner, 2011). There is no serological cross reactivity between E. canis and Anaplasma platys, and little cross reactivity, if any, with Rickettsia rickettsii, of Rocky Mountain Spotted Fever (Harrus and Waner, 2011).

Persistent titres may occur in persistent infections, reinfection, treatment failure, and an abnormal immune response. It has been speculated that dogs infected with vector-transmitted pathogens may develop persistent infection within systemic circulation and therefore may be more prone to develop autoimmune anti-nuclear antibodies (ANA) (Smith et al., 2004). It has been suggested that in persistent VBD infections, prolonged antigenic stimulation due to ineffective apoptosis or defective removal of cell debris by neutrophils and macrophages might lead to production of perinuclear antineutrophil cytoplasmic autoantibodies (pANCA) (Karagianni et al., 2012).

**Detection of parasites in blood and tissues**

Detection of piroplasms can be done morphologically, serologically or using molecular techniques. Cell culture isolation is expensive and time consuming. Serology may not differentiate acute from chronic phases, and testing need to be done in experienced diagnostic labs with stringent quality control measures. Cytological examination of thin blood films by light microscopy lacks both sensitivity and specificity and depends on the degree of parasitaemia. It might be difficult to detect Babesia through light microscopy when hemolytic anaemia or thrombocytopenia is present. However, the probability of detection of Babesia is high in smears made from capillary beds (ear tip, toe nail) or from cells beneath the Buffy coat. Detection in carrier dogs can be unrewarding due to very low, often intermittent parasitaemias (Irwin, 2009).

The morulae of Ehrlichia and anaplasma can be detected either in mononuclear cells, neutrophils or platelets, depending on the organism. The detection of morulae could be optimized by examining buffy coat smears (1000 oil immersion fields)(Harrus and Waner, 2011). Precautions must be taken not to confuse ehrlichial inclusions with platelets, lymphocytic azophilic granules, and phagocytes of nuclear material when examining blood smears. Cases may be erroneously diagnosed as lymphocytic leukemia as a large proportion of lymphocytes can show prominent azophilic granules, cleaved or indented cell nuclei with mature nuclear chromatin, consistent with large granular lymphocytes (Heeb et al., 2003).

Although the detection limit of light microscopy is approximately 0.001% parasitaemia for Babesia (Böse et al., 1995) and only 4% of blood smears for E. canis (Woody and Hoskins, 1991), Polymerase Chain Reaction (PCR) has a high sensitivity and specificity in parasite detection when there are approximately 50 organisms/ml (Birkenheuer et al., 2003) and 9 parasites/μl (Matsuu et al., 2005). However, fluctuating parasitemia can limit parasite detection by PCR testing. A one-step multiplex PCR has been developed for simultaneous detection of *Ehrlichia canis*, Babesia spp and *Hepatozoon canis*, from blood samples in a single reaction (Kledmanee et al., 2009). The primers used are specific to *E. canis* VirB9, Babesia spp 16S rRNA and *H. canis* 16S rRNA genes. The sensitivity in detecting *H. canis* in buffy coat, blood and bone marrow is high with PCR compared with cytotology using the same tissue (Otranto et al., 2011).

**General Clinical findings**

Critical determinants of infection severity are virulence of the organism, age, gender and the immune status of the patient. The records of the Veterinary Teaching Hospital (VTH) reveal that 34% of the dogs with TBD were less than one year old, and 52% of them were less than 6 months old. Males were at higher risk of infection with M:F ratio of 1.5. Approximately 28% of males was critically anemic (HCT <10%), compared with 14% females.

Rottweilers, Labradors, Boxers and cross bred dogs of any age were more susceptible for Babesiosis and Ehrlichiosis than other breeds. However, mostly young adults of German shepherds, Dobermann, and Pomeranians showed higher susceptibility. It was assumed that the cross breds were at a higher risk probably because the probability of getting infected is high as they were mostly free-ranging and also more exposed to dog bites during fights.

The degree of parasitemia depends on the immune status of susceptible dogs. When infected with the KR-1 strain of *B. microti*, the SCID (Severe Combined Immunodeficiency) mice and TCR knockouts (T cell receptor-beta-deficient mice) had shown to sustain severe parasitemia; IFN-γ deficient mice had developed a less severe parasitemia (Clawson et al., 2002). In contrast, the levels of parasitemia in JH-NULL mice which lack B-lymphocytes and antibodies were indistinguishable from the wild-type animals (Clawson et al., 2002). These data indicate that cellular immunity is critical for the clearance of *B. microti*.

Clinical signs and hematological changes, such as, hyperthermia, severe/critical anemia, thrombocytopenia, hypoalbuminemia, lymphadenopathy, hepatomegaly, splenomegaly and evidence of internal bleeding are not specific to a single tick-borne pathogen. The overall clinical and haematological findings propose a multi-system disease complex, and therefore, the findings should be carefully interpreted to avoid incorrect diagnosis and inappropriate treatment. Common clinical signs seen in TBDs are:

- Hyperthermia 104-106 °F (40-41°C), Sometimes hypothermia in Babesiosis (98.7°F)
- Anemia severe/critical, Evan's syndrome (IMHA/ IMTP)
- Leukocytosis/Leucopenia
- Thrombocytopenia with or without clinical bleeding such as, epistaxis, hematuria
- Hypoalbuminemia
Tachycardia (up to 80/min), elevated CRT
Tachypnoea (up to 80/min), dyspnoea, pulmonary oedema
Lymphadenopathy, splenomegaly, hepatomegaly, liver enzyme, jaundice, bilirubinuria
Occasional signs seen in Ehrlichiosis are;
- Neurologic (ataxia, seizures, paraparesis/tetraparesis [upper/lower motor-neuron deficits], stupor, vestibular disease)
- Polyarthritis (stiffness, swollen joints, reluctance to move [E. ewingii and A. Phagocytophilum])
- Ocular lesions (chorioretinitis, retinal detachment, uveitis)
- Vomiting, diarrhea

Systemic Findings
The vector-borne parasites cause severe damage to various organs in the host. The highest pathological grading have been seen in E. canis infected dogs followed by E. chaffeensis, A. platys and A. phagocytophilum, respectively (Nair et al., 2016). Chronic ehrlichiosis is associated with irreversible bone marrow destruction (Skotarczak, 2003). The infection status of E. canis and E. chaffeensis can be detected better with PCR testing of spleen and lymphnodes compared to A. platys and A. phagocytophilum (Nair et al., 2016). Detection of Ehrlichia DNA in tissues may not necessarily correlate with the presence of viable organisms, as Ehrlichial DNA can be found in blood or haemolymphatic tissues in dogs in which the infections are at sub-clinical level or has contributed to other disease conditions and masked by the presenting condition (Gal et al., 2008). It has been shown experimentally that Ehrlichial DNA could not be detected from blood or spleen after 9 and 60 days of treatment with doxycycline, respectively (Harrus et al., 2004). Therefore, it can be presumed that DNA from dead E. canis organisms gets probably cleared up in dogs and does not persist in the tissues for a long time after the elimination of infection by successful treatment or by an effective host immune response.

Anemia and Fever
Anemia caused by hemolysis, internal hemorrhage, or poor erythropoiesis could be critical leading to hypoxemia. The hemolysis could be intravascular and/or extravascular and either immune-mediated (IMHA) or non-immune mediated. Both intra and extravascular hemolysis lead to fever, icterus with elevated bilirubin. Ghost cells, autoagglutination and hemoglobinemia (cell-free plasma hemoglobin indicated by an elevated mean corpuscular hemolysis, MCHC), and hemoglobinuria are evidences of immune-mediated intravascular hemolysis. Erythrophagocytosis and spherocytes, indicating complete and partial phagocytosis, respectively, are evidence of immune-mediated extravascular hemolysis where hemolysis occurs in the mononuclear phagocytic system (MPS). Hemolysis due to non-immune mediation occurs as a result of oxidative damage, intensive lipid peroxidation due to generation of reactive oxygen species (ROS) molecules in parasitized erythrocytes, increased membrane permeability and decreased membrane potential. Malondialdehyde (MDA), an end product of ROS excreted in the urine, blood, and body fluids indicates oxidative stress and non-immune mediated hemolysis in B. gibsoni, B. canis and in experimental mixed infection of Ehrlichia canis and B. gibsoni (DVM360.com). Elevated MDA is a prognostic marker of disease severity and outcome.

In most occasions, a marked drop in HCT could be seen before parasitemia is detected in peripheral blood. This happens when erythrocyte destruction is greater than the degree of parasitemia indicating lysis of both parasitized and non-parasitized erythrocytes. The compensatory response to anemia by the bone marrow is evident by polychromasias indicating the presence of reticulocytes. The bone marrow response is classified as non-regenerative, or decompensatory, when nucleated erythrocytes (NRBC) are evident in peripheral blood, indicating the need to elevate the RBC volume by stimulating erythropoiesis or transfusing erythrocytes in order to alleviate hypoxemia. However, blood transfusions may not be possible in IMHA due to the presence of anti-erythrocytic antibodies which would lyse the transfused cells. Of the rickettsial organisms, E. canis cause the most significant drop in the HCT when compared with E. chaffeensis, A. phagocytophilum and A. platys (Nair et al., 2016). Samples with autoagglutination may record an elevated red cell distribution width (RDW).

The immune driven hypoproliferative anemia (Anemia of Inflammatory Disease, AID) could be another finding in Babesiosis and Ehrlichiosis. In AID, the availability of iron get reduced as a result of cytokine activation which inhibit duodenal Fe absorption, increase Fe uptake and retention in macrophages, reducing erythropoietin production in the kidneys, and directly inhibiting erythroid progenitor cells (Weiss and Goodnough, 2005). Bone marrow erythropoiesis can get impaired due to cytokine-mediated apoptosis, down-regulation of erythropoetin receptor expression on erythroid progenitor cells, poor expression of other prohaematopoietic factors, and low Fe availability for hemoglobin synthesis (Weiss and Goodnough, 2005). Hypoferremia despite adequate Fe stores is a host response to limit microbial access to Fe. This inhibition of pathogens due to limited access of iron, has been demonstrated in Ehrlichia (Park and Rikihisa, 1991).

When comparing the pathogens of the family Anaplasmataceae, pronounced anemia and reduction in PCV was seen in E. canis infections, and severe thrombocytopenia was manifested with the plateletrophic A. platys (Nair et al., 2016).

Vasculopathy, Hemoconcentration and Hypercoagulability
Following intravascular hemolysis, the decompartmentalized hemoglobin or cell-free plasma oxyhemoglobin causes irreversible oxidation of endothelial NO resulting in vasomotor instability and
vasculopathy (Minneci et al., 2005; Palmer et al., 1988; Rother et al., 2005). The NO regulates homeostatic vascular functions such as vasodilation, inhibition of platelet activation and thrombosis, inhibition of endothelial adhesion molecule and endothelin expression, and modulation of intimal and smooth muscle proliferation (Furchgott and Zawadzki 1980).

High mortality may occur as a result of loss of intravascular fluid due to increased capillary permeability, causing the extravascular protein-rich fluid compartment to raise leading to hemococoncentration and hyperviscosity, known as “red biliary”. This state will be clinically evident either as an absolute elevation of HCT from severe or critical anemia to over 30% or a normal HCT that is inappropriate for the degree of hemolysis (relative elevation of HCT) (Pardini, 2000).

Haemoglobin-mediated oxidative damage of endothelia during intravascular haemolysis is generally prevented by Hemopexin in plasma (Kuleš et al., 2014). Endothelial damage can also occur by reactive oxygen intermediates (ROS), activation of the complement cascade and cytokine activation on endothelia, as a result of autoaglutination, circulatory stasis and sequestration of parasitized cells. Endothelial phospholipase damage activates the coagulation cascade leading to the hypercoagulable state of disseminated intravascular coagulation (DIC) and formation of micro-thrombi. Thromboelastography (TEG) and thromboelastometry (ROTEM) can detect hypercoagulable states (Winberg et al., 2005). The TEG gives a graphical representation of clot formation and lysis, evaluating precoagulation, coagulation, and fibrinolysis.

The micro thrombi formed from DIC can lead to venous thrombosis, obstruction of vessels and tissue ischemia, particularly in the brain and lungs, thus producing clinical signs associated with pulmonary thromboembolism (PTE) and nervous system. The coagulation panel will reveal thrombocytopenia and hyperfibrinogenemia, increased circulating D-dimers, shortened PT or aPTT (Song et al., 2016). The consumption coagulopathy resulting from DIC will exacerbate hemorrhage caused by direct vasculopathy and thrombocytopenia, thereby increasing the risk of mortality due to hemorrhages. Plasma may reveal hypofibrinogenemia, elevated APTT, PT, FDP, D-dimer, and buccal mucosal bleeding time. The hemorrhaging will be clinically evident as epistaxis, hematuria, petechia, and melena as single or multiple manifestations. The survival for dogs with aortic thrombosis (ATh) or aortic thromboembolism (ATE) would be between 50% and 60%, and those with chronic clinical signs have a better prognosis than those acutely or severely affected (Williams et al., 2016). The prevalence of PTE in dogs is underestimated in necropsies, since thrombi in dogs lyse more rapidly than in humans (within 3 hours of death) due to greater plasminogen activator activity, greater platelet lytic activity, and secretion of plasminogen activator by the pulmonary endothelium (Goggs et al., 2009).

Thrombocytopenia
Severe thrombocytopenia is a consistent finding in the acute phase of Babesiosis. Thrombocytopenia, either constant or cyclic, is an almost consistent finding in Rickettsial infections, but it cannot distinguish Ehrlichial/anaplasma infections (Nair et al., 2016). Thrombocytopenia can be critical or severe, with or without clinical bleeding. Significant persistent thrombocytopenia is observed with platelet-trophic A. platis, and also E. canis and A. phagocytophilum infections even though cell tropisms for the latter two pathogens are monocytes and granulocytes, respectively (Nair et al., 2016). Thrombocytopenia from A. platis infection is a result of pathogen antigens, whereas thrombocytopenia in Ehrlichiosis is due to anti-platelet antibodies, but not due to anti-ehrlichial antibodies (Nair et al., 2016), because E. canis alters the immune system to overproduce natural anti-platelet antibodies with high affinity (Harrus et al., 1998; Harrus et al., 1996). Decreasing platelet counts correlate well with increasing anti-thromboocyte IFA titres and PCR positivity for E. canis DNA 16S rRNA, highlighting the importance of evaluating the platelet counts in suspected dogs (Harrus and Waner, 2011; Harrus et al., 1998; Harrus et al., 1996; Köster et al., 2015). It is important to rule out platelet agglutination in blood smears when evaluating the actual platelet count. Immune mediated thrombocytopenia (IMTP) occurring together with immune mediated hemolytic anemia (IMHA) is identified as the Evans Syndrome.

Evidence of bleeding without severe thrombocytopenia could be due to thrombocytopenia causing reduced platelet aggregation, reduced platelet adhesiveness, Interference in PF3 release and platelet migration inhibition causing reduced pseudopod formation.

Hypoxemia and Hypoperfusion
The pathophysiological sequelae induced by PTE result in hypoxemia, hyperventilation, and dyspnea. The arterial hypoxemia secondary to multiple abnormal ventilation: perfusion (V:Q) ratios in the affected lungs, can get complicated from interstitial and alveolar edema (congestive atelectasis) causing diffusion impairment, airway closure and alveolar collapse. An occlusion of >60% of the pulmonary vasculature will increase pulmonary vascular resistance (PVR) which will reduce the pulmonary arterial flow (Ebert et al., 1967). Reflex vasoconstriction secondary to alveolar hypoxia may also contribute to elevated PVR leading to pulmonary arterial hypertension (PAH) and increased right ventricular (RV) afterload, RV dilatation and dysfunction (Goggs et al., 2009). The reduction in left ventricular filling as a consequence of RV dilatation will decrease cardiac output leading to signs of forward failure (hypotension, cardiogenic shock). If the patient survives an acute crisis the residual pulmonary hypertension will lead to a long term backward failure resulting in hepatomegaly, ascites, and pleural effusion.
These mechanisms finally lead to severe hypoperfusion, concentrations. The ECG abnormalities were not deficit leading to severe hypoxemia and hypovolemia. Cardiac- Elevated cardiac troponin I (cTnI). The resulting hypoperfusion will demand a change cTnI concentrations, other than the ventricular maintain perfusion to vital organs and tissues, namely heart, lungs, brain, diaphragm, and intercostal muscles (Adachi et al., 1976). Such changes in the presence of restricted coronary blood flow and coronary sinus PO2 could be achieved by increasing myocardial O2 consumption and efficiency, and the cardiac work. The hemodynamic and metabolic changes in such patients would be bradycardia, hypotension, reduced cardiac index [CI = LV Q/ BSA (mL/min/m2)], reduced shock index (i.Shock =HR/SBP), mild changes in pulmonary capillary pressure (PCP) and central venous pressure (CVP), reduced mixed venous saturation (SVO2, percent O2 bound to HB in right atrium), reduced venous O2 pressure (PvO2), reduced O2 transport, while increasing O2 consumption (VO2), O2 extraction (TEO2), and serum lactate. The prognosis of such patients would be grave due to generalized edema and pulmonary edema (greater mortality), ultimately leading to cardiac failure and multiple organ dysfunction. Such patients may show evidence of hepatic dysfunction (hyposalbuminemia, markedly elevated ALT, elevated bilirubin, icterus and bilirubinuria), impaired gas exchange & respiratory dysfunction (dyspnea, tachypnoea up to 80/m), pulmonary edema, acute respiratory distress syndrome (ARDS), and renal azotemia which will drastically increase the risk of mortality (Harison et al., 2012). The systemic vasoconstriction resulting from vasculopathy and hypovolemia will further impair renal function leading to renal failure.

Clinical Biomarkers of tick-borne diseases
Clinical biomarkers signify multisystemic pathologies (Diniz et al., 2008; Köster et al., 2015; Koutinas et al., 2012).

Blood Profile- IMHA. Hemolytic anemia, Thrombocytopenia, Hemoconcentration Coagulation profile- Thrombocytopenia, consumption coagulopathy (↑APTT, PT, D-dimer) Hemochemical abnormalities - metabolic acidosis & respiratory alkalosis, Hyperlactatemia, Hyperbilirubinemia of >170 μmol/L, elevated ALT Cardiac- Elevated cardiac troponin I (cTnI) concentrations. The ECG abnormalities were not associated with disease severity, outcome, or plasma cTnI concentrations, other than the ventricular premature complexes associated with high cTnI. Respiratory-Pulmonary edema.

For acute and chronic diseases of Ehrlichia and anaplasma, the carriers can be categorized according to results of multiple tests, as shown in Table below. Cross-reactions are common in serological tests such asIFA, dot-ELISA (“Immunocomb”). Nested PCR (nPCR) by 16S rRNA amplification, detect DNA earlier than serology, and cross-reactions are uncommon. A p30-based nested PCR assay has been developed for detection of E. canis in both dog and ticks (Stich et al., 2002). The coombs’ test cannot diagnose IMHA because it lacks sensitivity.

Most dogs recover from clinical signs with treatment but no single drug had been successful in completely eliminating the organisms, and thus requiring adjunctive treatment, including O, therapy, immunosuppressants, antithrombotics, antimicrobials and fluid. Anti-babesiacidal drugs act by many different mechanisms. Atovaquone (ubiquinone analog) block mitochondrial electron transfer leading to reduced energy production for parasites. Berenil (Diminazene aceturate) interferes in aerobic glycolysis (reduce energy production) and multiplication (interact with DNA minor groove). The Berenil resistant genes (CYTb) can transfer during pathogen multiplication, and the proliferation potential and the degree of parasitemia is low in Berenil resistant Babesia strains. Berenil toxicity can occur from overdosing, repeated dosing, and even with recommended doses on Babesia negative animals. The anticholinergic anthelmintic, Imidocarb (carbanilide) can be used in most tick-borne diseases. Drug combinations have shown to be either effective or suppress parasitemia below the limit of detection, thereby reducing the transmission of protozoa through vectors.

Ehrlichia organisms survive and multiply in the infected phagocyte by their ability to inhibit the fusion of the phagosomesomes (Park and Rikihisa, 1991; Wells and Rikihisa, 1988). Treatment of Ehrlichiosis with doxycycline, an antibiotic which inhibits prokaryote protein synthesis, restores phagosomesomes fusion (Wells and Rikihisa, 1988).

Table 1. Interpretation of laboratory findings in Ehrlichiosis

<table>
<thead>
<tr>
<th>Serology</th>
<th>nPCR</th>
<th>WBC</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Leukocytosis</td>
<td>Acute disease (Morulae)</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Leukocytosis</td>
<td>Acute disease</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Pancytopenic</td>
<td>Chronic disease</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td>Persistent titres (Carriers/Treated)</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

The author acknowledges the clinical and laboratory contributions of Dr. WCR Fernando, Ms. MRCK Mallawa of the Veterinary Teaching Hospital.

REFERENCES


Pardini A.D. (2000). The pathology and pathogenesis of canine cerebral babesiosis (Dissertation). Onderstepoort, Faculty of Veterinary Science, University of Pretoria


Agricultural and Environmental Medicine. 10, 137-142
https://doi.org/10.1016/S0304-4017(00)00407-6
ORIGINAL ARTICLE

ASSESSMENT AND COMPARISON OF URINARY PROTEIN: CREATININE RATIOS OF HEALTHY DOMESTIC DOGS AND WORKING DOGS IN KENNELS DIVISION IN SRI LANKA POLICE FOR EARLY DETECTION OF RENAL DISEASE

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Summary: Healthy dogs usually excrete small amounts of protein in urine. Persistently high proteinuria is usually a marker of kidney disease. The urinary protein: creatinine ratio (UPC) is used in quantifying urine protein excretion diseases, guiding recommendations for monitoring and treatment of kidney diseases and evaluation of prognosis. However, use of UPC ratio has not been well established/used to diagnose renal diseases in Sri Lanka. Therefore, for the first time in Sri Lanka, we used this method to detect UPC ratio of healthy dogs (n=51). Once we established the UPC ratios of healthy dogs, UPC ratios were measured in working dogs (n=45) in Kennels Division of Sri Lanka Police, since renal diseases are a common cause of mortality in these dogs. In control group, the mean UPC ratio was 0.06±0.05. This was similar to the UPC ratio of tracking group among working dogs. Two out of 22 dogs used for explosive duties had UPC ratio within the borderline proteinuric stage (0.2-0.5) according to the staging system implemented by the International Renal Interest Society (IRIS). In the narcotic group 2 out of 12 dogs were borderline proteinuric and one dog was proteinuric. In this study, we have successfully established the measurement of UPC ratio to detect proteinuria in Sri Lankan dogs and have identified dogs that are susceptible to renal diseases.

INTRODUCTION

Proteinuria is one of the risk factors of renal diseases. In addition to this, proteinuria is used as a predictor of end organ damage (Barnas et al., 1997). Early and accurate detection of persistent renal proteinuria is of high importance to reduce mortality in both dogs and cats due to renal damage (Littman, 2011; Wehner et al., 2008). By detecting acute renal disease early in its course, appropriate intervention can be made to arrest or at least to attenuate renal damage and the development of acute renal failure. Similarly, by detecting chronic renal disease before the onset of renal azotemia and chronic renal failure, appropriate intervention can be made to stabilize renal function or to slow its progression (Jacob et al., 2005). Once a complete diagnosis is made, therapeutic and management plans can be arranged to manage the disease. The urine protein creatinine (UPC) is the gold standard tests to quantify and monitor proteinuria in dogs. UPC ratio detects persistent proteinuria and aid in making clinical decisions and monitoring response to therapy. UPC values recommended by American College of Veterinary Internal Medicine (ACVIM) (Lees et al., 2005) and International Renal Interest Society (IRIS) for azotemic and non azotemic dogs provide guidance in monitoring, diagnosing, and treating dogs and cats with renal disease (Brown et al., 2013; http://www.iris-kidney.com). We have observed higher mortality rates in working dogs in Police and armed forces in Sri Lanka, due to renal diseases. Therefore, the primary goal of this study was to establish the reference for UPC ratio for dogs in Sri Lanka, and to compare UPC ratios of working dogs in Sri Lanka Police to detect the dogs with possible renal diseases early.

MATERIALS & METHODS

Design and study population

Fifty one client-owned, apparently healthy dogs presented to the Veterinary Teaching Hospital (VTH), University of Peradeniya, identified as reference group, were used to establish the UPC ratio. These dogs were included in the study only after signed consent had been received. Their average age was 2.8 years (range 1-10 years). All the working dogs (n=45) except three puppies (< 6 months) and six dogs attached to Presidential Security Division of Sri Lanka Police were used in this study. Their average age was 4.3 years (range 2-8 years). This group comprised of dogs assigned for explosive detection, narcotic detection and tracking duties. Dogs were enrolled for this study using the following inclusion criteria: (1) more than six months of age, any breed, gender, size and (2) were either healthy animals, or working dogs with or without underlying disease/s that could induce proteinuria.

http://doi.org/10.4038/slvj.v63i2.10
A single random mid-stream sample of 10 ml urine (Monroe et al., 1989) was collected aseptically into sterile tubes from each animal by catheterization and transported in ice immediately after collection. Urine samples from animals with active sediment were excluded from the study. An inactive urine sediment for this study was defined as <5 WBC/ hpf, <20 RBC/ hpf, and no visible bacteria (Duffy et al., 2015). Humantrol quality control sera (Human GmbH-Max-Plank-Ring, Germany) were used to check the experimental procedures.

Blood samples
Venous blood sample (2.5 ml each) was collected into EDTA tubes from cephalic vein. Plasma was separated within 30 minutes from collection. Hematologic and plasma analytes were determined within 12 hours with standard methods. Samples were analyzed for Full Blood Count, Blood Urea Nitrogen (BUN), plasma creatinine, total plasma protein and albumin according to the standard diagnostic laboratory procedures established at the VTH. Blood smears were prepared and checked to exclude tick-borne parasitic infections.

Urinalysis and UPC measurement
Urine samples were used to estimate specific gravity, protein and creatinine concentrations, and subjected to microscopic sediment evaluations, within 12 hours of collection (Rosi et al., 2012). Samples were assessed visually for evidence of debris, or gross discoloration (Vaden et al., 2004). Urine specific gravity was measured using a refractometer. Multistix Uripath strips were used according to the manufacturer's guidelines to detect glucose, bilirubin, ketones, specific gravity, occult blood, pH, protein, nitrite and leucocytes in urine by assessing the colour change at recommended times after immersing the dipstick in the sample of urine. Urine sediment, 0.5 ml, harvested by centrifuging 5 ml of urine sample at 2000 rpm for 5 minute was evaluated microscopically at low power (10x) for epithelial cells, casts and at high power (40x) for leukocytes, erythrocytes, bacteria and crystals.

Urinary protein concentration
Total protein concentration (mg/dl) in urine was measured in the supernatant obtained after centrifugation by Pyrogallol red-molybdate method (Johnson et al., 1999) on a semi-automated biochemistry analyzer (Erba Mannheim, ERBA Diagnostics, North Miami Avenue Miami, U.S.A). In this method pyrogallol red/molybdate formed a red complex in the presence of proteins and the colour was directly proportional to the protein concentration.

Urinary creatinine concentration
Urine creatinine concentrations (mg/dl) were measured using RANDEX Monza reagent with the semi automated chemical analyzer using a modified Jaffe method (Bartels, 1972). In this colorimetric method creatinine in alkaline solutions reacts with picric acid and forms a coloured complex which is proportional to the creatinine concentration.

Blood Urea Nitrogen concentration
Blood urea nitrogen concentration (mg/dl) was measured using urease-Berthelot method using RANDEX reagent with the semi automated chemical analyzer. In this colorimetric method ammonia which is produced by urea in serum in the presence of urease is measured photometrically.

Albumin concentrations in plasma
Albumin concentrations (mg/dl) in plasma were measured using RANDEX Monza reagent with the same automated chemical analyzer. In this colorimetric method the measurement of serum albumin is based on its quantitative binding to the indicator bromocresol green.

Classification
IRIS staging classification was used to classify UPC ratios <0.2 as nonproteinuric, =0.2 and <0.5 as borderline proteinuric and =0.5 as proteinuric. Clinical response category based on the ACVIM consensus statement (Lees et al., 2005) was also assessed with the serum creatinine concentrations. For nonazotemic category, “no action” was taken for dogs with UPC <0.5; monitoring for kidney function was advised for those who had UPC =0.5 and further investigations to assess the kidney function were recommended for dogs with UPC =1 and <2 and medical intervention for UPC with =2. Azotemic dogs with UPC <0.5 was considered as “nonintervention” category and UPC =0.5 was considered as “intervention” category for management of the condition.

Health records of Working dogs in Kennels division of Sri Lanka Police
In a retrospective study, carried out in parallel to this study, we collected data of working dogs in the police for past two years.

Statistical Analysis
Data are expressed as mean±SD, and 95% CIs were calculated. All analyses were performed with MINITAB® Release 14.1 software. Results obtained for control group and working dogs were compared by using the two sample t-test and ANOVA. The Regression test was used to assess a possible relationship between UPC ratio and other factors. Values of $p<0.05$ were considered significant.

RESULTS
Population
Urine samples were obtained from 51 client-owned dogs. Samples from all of these dogs were included in the study, since they had inactive urine sediment. Number of dogs in the kennels division of Sri Lanka Police had variable degrees of active urine sediment and the samples which were compatible with our inclusion criteria were included in the study (Figure 1).
Urinalysis of control group

Average UPC ratio of 51 healthy animals in the control group was estimated to be 0.06±0.05 (Table 1).

Results obtained using health records of working dogs in kennels division of Sri Lanka Police showed that twenty one deaths have been reported and necropsies performed at the Division of Pathology of the Department of Pathobiology, and Division of the Diagnostic Pathology of the VTH, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya. Necropsy reports indicated that the highest number of deaths was due to renal failure (33.3%). In addition to this, haemoparasitism (24.7%), heart failure (19%), multi organ dysfunction (9.5%), toxicosis (9.5%) and lymphosarcoma (4%) were associated with the deaths of working dogs in police.

Results of UPC ratios in control group and working dogs

Mean and standard deviation of UPC ratio in healthy dogs was 0.06±0.05 (n=51). Mean and standard deviation of UPC ratio in dogs in explosive (n=22), narcotic (n=12) and tracking (n=9) duties were 0.09±0.09, 0.15±0.26 and 0.05±0.06 respectively. One way ANOVA–revealed a significant difference between mean UPC values in control group and working dogs (p<0.05, R-Sq=9.91%). Two sample t-tests were used to compare UPC values between groups. Results showed that there were significant differences (p<0.05) between UPC ratios in dogs in the control group vs explosive and narcotic groups. There was no significant difference (p>0.05) in UPC value of dogs in the control group vs tracking group.

One of the key objectives of this study was to screen the renal diseases of working dogs in the kennels division of Sri Lanka Police. Therefore, rather than comparing the mean UPC ratios between groups, we compared UPC ratios of individual animals in the study population with the IRIS standards recommended by ACVMA (Figure 2). In the narcotic group, one animal was proteinuric but nonazotemic. According to the ACVIM co.n.sensus statement this animal was subjected for monitoring of kidney function. Two animals in the narcotic and two in the explosive groups were borderline proteinuric hence no action was taken (http://www.iris-kidney.com/) (Table 2).
An increase in urinary protein excretion is a widely accepted tool in the detection, diagnosis, and management of renal disease (Price et al., 2005). The UPC ratio is the gold standard test to quantify proteinuria in dogs (Harley and Langston, 2012). However, in Sri Lanka there had been no evidence of using this test to stage renal diseases in dogs. Therefore, in this study we were able to successfully assess and establish UPC ratios in a cohort of healthy dogs in Sri Lanka. All the animals sampled in the control group were nonproteinuric and had no sign of active urine sediment. The differences in UPC values among the animals in the control group would have been due to the time of the day of urine samples collected as one report suggested that the first morning specimens are preferred to detect protein excretion (Xin et al., 2004).

However, there are several reports supporting the idea that use of random urine samples for the measurements of UPC ratios work accurate and more practical (Newman et al., 2000; Price et al., 2005).

Study of necropsy records (from 2012-2014), of working dogs in Kennels division of Sri Lanka Police supports the fact that renal failure is the major cause for deaths. Bringing up a working dog to their working capacity from the time of importation is quite an expensive task. Therefore, detecting renal function at early age is of high importance for successful monitoring and therapeutic interventions. In our survey, we have found that even young working dogs (age 2-5 years) have died of renal disease. Chronic renal failure has been the cause of death (~71%) in many of these animals diagnosed with renal disease. Although, the cases of renal failure are presented towards the latter part of their illness, proper
screening of kidney function in dogs using UPC ratio would significantly reduce the mortality. It is not just the animals with kidney disease that should be screened for proteinuria. According to the author’s experience, working dogs in the Sri Lanka police are very prone to have conditions or diseases such as drug reactions, hyperadrenocorticism, acute renal failure, viral disease, immune mediated diseases, tick-borne diseases, exogenous steroid use, and urogenital diseases that may contribute to proteinuria. This is further supported by a report of Harley and Langston (2012). UPC ratios of working dogs are affected by number of other factors such as dietary protein content, exercise, and hyperthermia (Schaefter et al., 2007; Mustafa et al., 2011). Previous studies reported that cage confined animals had significantly higher UPC ratios compared to unconfined animals, and stress in these animals would have contributed for the difference (McCaw et al., 1985).

When we compared UPC values of dogs in the tracking group and control groups, there was no significant difference. This could possibly be due to the fact that they are used for minor duties such as tracking crimes where the chances for inhalation/ingestion of nephrotoxic agents are low.

There was one animal in the explosive group who was proteinuric, but nonazotemic. According to the ACVIM consensus statement this subject was monitored for kidney function. Two animals in the narcotic and two in the explosive groups were borderline proteinuric hence no action was taken. Therefore, advantage of the method we established in the Diagnostic laboratory of the VTH, would be to routinely screen dogs for renal diseases. Nine months old puppies in the explosive group are trained to sniff explosives such as Tri NitroToluene (TNT), Research Department Explosive (RDX/Cyclonite), Dynamite, plastic explosive, Pentaerythrite Tetranitrate (PETN), NH₄NO₃, Detcord, electrical detonator, non-electrical detonator every day for four hours in the morning and one hour in evening which continues throughout their life time and this chronic exposure makes them prone to renal injury.

In the narcotic group, one animal was proteinuric but nonazotemic and two animals were borderline proteinuric. This could possibly be due to the fact that they are trained to sniff narcotics such as heroin, marijuana, cocaine, toddy, ganja and arrack. It has been reported from humans who abuse narcotics are more prone to be affected with renal failure resulting from chronic glomerulonephritis and hypertensive nephrosclerosis (Xiqian et al., 2015).

In summary, this study assessed and established UPC ratios in a cohort of healthy dogs in Sri Lanka. These values were in agreement with the UPC ratios reported in IRIS staging system. The degree of difference in UPC values in working dogs of the Sri Lanka police warrants attention of the authorities and clinicians regarding clinical decisions about diagnostic, monitoring, or therapeutic plans. Nevertheless, screening of these high risks groups of working dogs for their kidney function and also use of UPC measurement in other dogs as a screening test would assist clinicians in detecting possible kidney diseases in advance.

ACKNOWLEDGEMENT

This study was carried out with the financial support provided by the University Research Grant (RG/2013/18V). The authors thank Dr. M. L. A. N. R. Deepani for help with statistical analysis of data, Mrs. M.R.C.K. Mallawa and Ms. A.H.M.W.S Aberathna for assisting in conducting experiments and Sri Lanka Police and Clients of dogs who volunteered to provide samples of animals for the research project.

REFERENCES


Wijayawardhane et al. 2016


https://doi.org/10.2460/ajvr.73.6.779

https://doi.org/10.1111/j.1939-1676.2011.00829.x

https://doi.org/10.1016/j.cca.2004.06.019

https://doi.org/10.1016/j.cccn.2004.06.019
BODY CONDITION SCORE IN LARGE PURE BRED DOGS: A PRELIMINARY STUDY ON AGREEMENT BETWEEN OWNER'S PERCEPTION AND SCIENTIFIC EVALUATION

M. Jagatheesan, D. D. N De Silva and H. M. H. S Ariyarathna

Department of Veterinary clinical Science, University of Peradeniya

SUMMARY: Obesity is a severe health issue among companion dogs. It may seriously impair the quality of a dog's life predisposing to many other disease conditions. Incorrect assessment of the body condition (BC) of their pets by the dog owners has been identified as an obstacle which would prevent early intervention in canine obesity. This study was designed to assess the degree of agreement between the dog owner's perception of their pet's BC and the scientific assessment of body condition score (BCS). Eighty-two large pure bred dogs were included in the study. Scientific evaluation was performed using 1-5 BCS system proposed by Royal Canin™ (1=emaciated, 2=thin, 3=ideal, 4-over weight, 5- obese). Following the scientific evaluation the owners were asked to assess their pet's BC using a grading system which included appropriate lay terms to describe the 1-5 scoring positions in the scientific method (1=very thin, 2=thin, 3=ideal, 4=fat, 5=very fat). In addition the knowledge of necessity of feeding adjustments and exercise, health risk of obesity and awareness of scientific BC evaluation methods were evaluated using a short questionnaire. Agreement between the owner's perception and scientific method was analyzed using Fleiss' kappa test. Misperception occurred among 31.71% (26/82) of the owners and there was only a fair agreement (K=0.374321, P<0.05) between two evaluation methods. Interestingly owner misperception about the body condition existed not only in the BCS=4/ Overweight and BCS=5/ Obese groups but also in BCS=2/ Thin group as well. Despite the good knowledge on health risk of obesity and need of exercise among the owners, under estimation was common in BCS=4/ Overweight (40%, 8/20) and BCS =5/ Obese (100%, 5/5) groups which might be a sequela to owner's misperception. In the BCS=2/ Thin group there was a prominent overestimation 3/5 (60%). Only 12.1% of the owners were aware of existence of scientific body condition evaluation methods. Current findings are suggestive of the importance of modifying the owner's perception about their pet's body condition and the necessity of the adequate veterinary intervention through client communication about pet feeding and exercising.

INTRODUCTION

The prevalence of obesity in dogs has increased in recent years (Olsen, 2011). Estimation about the incidence of obesity in various part of the world in dogs reveals that 18%-44% of the population is obese (Anderson, 1973; Edney & Smith, 1986; Kronfeld et al., 1991; Lund et al., 1999; Mason, 1970; Robertson, 2003; Sonnenschein et al., 1991). Excessive deposition of adipose tissue sequelae to positive energy balance is the cause of obesity (Coliardi et al., 2006; German, 2006; German et al., 2007; Gosselin et al., 2007; Laflamme, 2006; McGreevey et al., 2005). Predisposing factors of obesity include genetics, increased daily energy intake, reduced physical activity level, certain medications (e.g. glucocorticoids) and diseases (e.g. hypothyroidism) (Czirjak & Chereji, 2008; German, 2006). Neutering/spaying also suggested to be a predisposing factor due to the effect of reduced energy requirement (Fettman et al., 1997; Flynn et al., 1996; Jeusette et al., 2006; Jeusette et al., 2004; Root et al., 1996). It could deteriorate the quality of life in dogs as well shorten the life span by predisposing to conditions such as cardiorespiratory diseases (Ettinger & Feldman, 1995), osteoarthritis (Impellizeri et al., 2000), chronic inflammation, diabetes mellitus (Ettinger & Feldman, 1995), and neoplasia (German, 2006; Lund et al., 1999).

There are various scientific methods used to assess the body condition of dogs (German, 2006; Mawby et al., 2004). They are classified into two different categories as research techniques and clinical techniques. Research techniques include chemical analysis (D2O), densitometry, Dual Energy X-Ray Absorptiometry (DEXA), ultrasound and electrical impedance. Clinical techniques include measuring body weight and morphometric methods (BMI: Body Mass Index, BCS: Body Condition scoring) (German, 2006; Laflamme, 1997a; 1997b; Mawby et al., 2004). Clinical methods are comparatively quick, inexpensive and non- invasive (German, 2006). BCS is one of the common reliable clinical method (German et al., 2007). Four different types of scales are in use [5 scale and 6 scale (German, 2006), 7 scale and 9 scale (Laflamme, 1997b; 1997a). Body condition is determined using visual assessment and palpation which has a correlation with the degree of subcutaneous fat deposition, abdominal fat and condition of superficial muscles (German, 2006). Despite the existence of many methods, the owner's perception about the body condition of their canine companion plays a
significant role to maintain an ideal BC. Some studies conducted in UK revealed that under estimation of BC in pet dogs is quite common among the dog owners (Courcier et al., 2009; Eastland-Jones et al., 2014). Research to investigate the causes of misperception is important to maintain a healthy and ideal dog. In Sri Lanka there are no published studies on this topic. The objective of this study was to assess whether there is any significant difference between the owner's estimation and the scientific BC evaluation (1-5 scale system, Royal Canin™). In addition the knowledge and attitudes on feeding practices, necessity of exercise, awareness of health risks of obesity and awareness of scientific body condition evaluation methods were also assessed.

MATERIAL AND METHODS

The survey was carried out in the Veterinary Teaching Hospital, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya and in a Private Veterinary Practice in Colombo over a period of one month. Owner's consent was obtained prior to the assessment. Inclusion criteria for the study were, large purebred dogs older than 6 months (after sexual maturity) displaying breed characteristics specified for the relevant breed (Fogle, 2002) and (RSPCA 2012). The dog owners were requested to assess the body condition of their dog according to the provided guidelines. First the owners were requested to select an appropriate term out of the five lay terms provided which they think suitable for their pets. The used terms (very thin, thin, ideal, fat, very fat) represented the 1 5 scoring positions in the Royal Canin™ BCS system respectively. Following the assessment attitudes on necessity on diet modifications for overweight/obese dogs (Q2 in Table 4), necessity of exercise to prevent obesity in dogs (Q3 in Table 4), overfeeding in purebred dogs (Q4 in Table 4), awareness of health risk of obesity (Q5 in Table 4) and knowledge about scientific body condition evaluation methods other than body weight (Q6 in Table 4) were assessed using a short questionnaire. Scientific body condition evaluation was performed by a veterinarian using the five scale (1-5) scoring system provided by Royal Canin™ (Table 1). A score was determined for each dog using visual cues followed by palpation (1=emaciated (very thin), 2=thin, 3=ideal, 4=over weight (fat) 5=obese (very fat). In short haired dogs both the visual assessment and palpation helped to determine the fat deposition around the ribs, vertebral column, abdominal tuck and waist. In long haired dogs the determination of BCS heavily relied on palpation that was done to ensure the fat deposition around ribs, vertebral column, abdominal tuck and waist to correctly determine their true body condition. The information on gender and body weight were collected for the purpose of record keeping. The level of agreement between two assessments was interpreted according to the calculated kappa value (Fleiss’ Kappa test) using Minitab® 17 software (Table 2). To analyze the overall agreement, a kappa value was calculated for the whole group using the two sets of ratings produced by two assessment methods. Agreement within the groups was assessed using the kappa values calculated for each BCS group.

RESULTS AND DISCUSSION

A total number of 82 dogs were evaluated. Dog breeds included were Labrador retriever, German shepherd, Golden retrievers, Rottweiler, Doberman, Great Dane, Rhodesian Ridgeback, Boxer, Bullmastiff, Alaskan Malamute and Hungarian pointer. Most frequent dog breeds were Labrador retriever (34%, n=28) and German shepherd (23%, n=19).

Overall Kappa score of 0.37 (p <0.05, 95% CI) (Landis & Koch, 1977) indicated an overall fair agreement between owner's assessment and scientific BCS evaluation. Owner's assessments did not aligned with the scientific assessment in 31.7% dogs (26/82).

For further detailed analysis, dogs were divided in to five groups based on the body condition score obtained from the scientific method (Group 1/BCS-1, Group 2/BCS-2, Group 3/BCS-3, Group 4/BCS-4 and Group 5/BCS-5). As there were no dogs in the Group 1/BCS-1 the particular group was not

Table 1: Royal Canin™1-5 body condition scoring system

<table>
<thead>
<tr>
<th>BCS Assessment criteria</th>
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<tbody>
<tr>
<td>1 Ribs, spine, pelvic bones easily visible</td>
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<tr>
<td>Obvious loss of muscle mass</td>
</tr>
<tr>
<td>No palpable fat on rib cage</td>
</tr>
<tr>
<td>2 Ribs, top of vertebrae, pelvic bones visible</td>
</tr>
<tr>
<td>Obvious abdominal tuck (waist)</td>
</tr>
<tr>
<td>No palpable fat on rib cage</td>
</tr>
<tr>
<td>3 Ribs, spine, pelvic bones not visible but easily palpable</td>
</tr>
<tr>
<td>Obvious abdominal tuck</td>
</tr>
<tr>
<td>4 Ribs, spine, pelvic bones palpable with difficulty</td>
</tr>
<tr>
<td>Abdominal tuck absent</td>
</tr>
<tr>
<td>Fat deposits obvious on spine base of tail</td>
</tr>
<tr>
<td>5 Massive fat deposits on thorax, spine and base of tail</td>
</tr>
<tr>
<td>Obvious abdominal distention</td>
</tr>
</tbody>
</table>

Table 2: Statistical Analysis

<table>
<thead>
<tr>
<th>Kappa value</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>&lt;0</td>
<td>Poor agreement</td>
</tr>
<tr>
<td>0.01 0.20</td>
<td>Slight agreement</td>
</tr>
<tr>
<td>0.21 0.40</td>
<td>Fair agreement</td>
</tr>
<tr>
<td>0.41 0.60</td>
<td>Moderate agreement</td>
</tr>
<tr>
<td>0.61 0.80</td>
<td>Substantial agreement</td>
</tr>
<tr>
<td>0.81 1.00</td>
<td>Almost perfect agreement</td>
</tr>
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Fleiss’ Kappa test interpretation
considered for further analysis. The agreement between two assessments within individual groups was also determined using the same statistical method (Fleiss's kappa test).

Group 2/BCS=2 (n=5) had a kappa score of 0.36 (95% CI, p<0.05) indicating a fair agreement between the two raters. Even though there was a fair agreement between two assessments, a considerable degree (60%, Table 3) of overestimation existed. However due to the small sample size it is difficult to attempt to a conclusion that this is a significant finding. In this group only 20% of the owners (1/5, Table 4) believed that feed modifications are useful. However the owner's awareness about necessity of exercise and health risk of obesity were satisfactory (Table 4). Sixty percent (60%, Table 4) of the owners in this group did not practice excessive feeding in purebred dogs. Only 40% (Table 4) of owners were aware about scientific methods of evaluating body condition in dogs.

Group 3/BCS 3 the largest group (n=52) had a kappa score of 0.44 (95% CI, P<0.05) which indicated a moderate agreement between the two assessments. However, in this group also only 13.5% (Table 4) of the owners were aware about scientific evaluation methods for assessment of body condition in dogs. Even though only 13.5% (Table 4) of the owners were aware about scientific body condition evaluation methods, 90.4% (Table 4) of them were aware about the health risk of obesity and necessity of exercise (Table 4). A considerable proportion (40.4%, Table 4) of owners strictly rejected excess feeding in purebred dogs. This might have played an important role in maintaining an optimum BCS even in the absence of knowledge about a scientific BCS evaluation method. Moreover, the owner's awareness on necessity of exercise and the awareness on the health risk of obesity might have contributed substantially to maintaining these dogs in ideal body condition. However, 34.6% dogs had been overfed in this group which might be due to the lack of knowledge about a scientific evaluation method.

Group 4/BCS 4 (n=20) carried a kappa score of 0.38 (95% CI, P=0.05) which indicated a fair agreement between the two assessments. All dogs in this group were overweight according to the scientific evaluation. Interestingly majority of owners in this group (95%, Table 4) were aware of the health risks of obesity. Moreover 80% (Table 4) of the dog owners accepted that exercise is important. However only 5% (Table 4) of the dog owners were aware about an existence of scientific body condition evaluation methods other than body weight. Therefore, had they provided with a correct body condition scores assessed following a scientific method, they might have attempted to modify feeding practices and exercise to reduce the risk of being overweight. However, in spite of the awareness of the risk of obesity and necessity of exercise, 40% (Table 4) of the dog owners believed that purebred dogs need excessive feeding and only 35% (Table 4) of the owners accepted the need to modify the feed. Therefore, it is necessary to educate the pet owners that feed requirement in dogs does not depend on the breed but according to the energy expenditure and physiological status of the dog.

Group 5/BCS=5 (n=5) had a kappa score of 0.81 (95% CI, p<0.05) indicating a fair agreement between the two methods of assessment. According to the scientific evaluation all the dogs in this group were obese and dietary intervention and exercising is essential as they are already predisposed to various health issues (pressure wounds n = 3, exercise intolerance n = 4, infertility = 1). Interestingly under estimation by the pet owners was 100% (Table 4) even though all the owners were aware of the health risk of obesity as well as the need for exercise. Sixty percent 60% (Table 4) of the owners were willing to modify feed. Excessive feeding of purebred dogs was practiced by 40% (Table 4) of the owners while 40% (Table 4) of the owners disagreed with that. None of the dog owners in this group were aware of scientific body condition evaluation methods other than body weight (Table 4). Therefore, misperception of the body condition of their pets might be an important contributory factor for obesity in this group. However only 5 dogs were in this study and it is necessary to have a larger group to identify the consistency and significance of these findings.

In the groups 4 and 5 despite the awareness of health risks and necessity of exercise, dogs were overweight/obese. One possible explanation for this is existence of underlying disease conditions such as hypothyroidism, cardiac diseases and arthritis, which may predispose the dogs to obesity or interfere with exercising. However, the investigation of these possible underlying factors is beyond the scope of this study. Neutering also known to

<table>
<thead>
<tr>
<th>Group</th>
<th>Overestimated</th>
<th>Correct</th>
<th>Underestimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2/BCS 2 (n=5)</td>
<td>n=3 (60%)</td>
<td>n=2 (40%)</td>
<td>-</td>
</tr>
<tr>
<td>Group 3/BCS 3 (n=52)</td>
<td>n=6 (11.5%)</td>
<td>n=43 (82.7%)</td>
<td>n=3 (5.8%)</td>
</tr>
<tr>
<td>Group 4/BCS 4 (n=20)</td>
<td>n=1 (5%)</td>
<td>n=11 (55%)</td>
<td>n=8 (40%)</td>
</tr>
<tr>
<td>Group 5/BCS 5 (n=5)</td>
<td>n=0 (0%)</td>
<td>n=0 (0%)</td>
<td>n=5 (100%)</td>
</tr>
</tbody>
</table>

These results are formulated from the answer to the question 1 in the provided questionnaire. There were no candidates in the Group 1/BCS 1.
Table 4: Attitudes and knowledge on feeding practices, necessity of exercise, obesity and scientific methods of evaluating body condition in dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>Q6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y</td>
<td>N</td>
<td>DK</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>BCS-2 (n=5)</td>
<td>20%</td>
<td>60%</td>
<td>20%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>BCS-3 (n=52)</td>
<td>23.1%</td>
<td>61.5%</td>
<td>15.4%</td>
<td>90.4%</td>
<td>7.7%</td>
</tr>
<tr>
<td>BCS-4 (n=20)</td>
<td>35%</td>
<td>60%</td>
<td>5%</td>
<td>80%</td>
<td>15%</td>
</tr>
<tr>
<td>BCS-5 (n=5)</td>
<td>60%</td>
<td>40%</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

ACKNOWLEDGEMENT


RESULTS OF THE STUDY

Results of the study reveal that even though some of the pet owners are aware about the health risks of obesity they do not attempt for necessary diet modification and exercise due to improper perception of their pet's body condition. Therefore, current findings indicate the importance of a scientific method (based on standard criteria) in pure breed dogs to minimize obesity-related health risks. In a previous study conducted in UK (Courcier, et al. 2009), the underestimation of the body condition of dogs (n=1) from the obese group was observed in our study (Table 2). However, a study demonstrated an overall 21.9% underestimation in the body condition of dogs (Courcier, et al. 2009). Our study indicates that the body condition of dogs in the overweight group was 52% (n=2) and in the obese group 77% (n=6) underestimated the body condition of their dogs in our study (Table 2). Interestingly, 44.1% of owners underestimated the body condition of their dogs (Courcier, et al. 2009). In our study, 77% of owners underestimated the body condition of their dogs (n=231). Fifty-three percent of overweight dog owners had underestimated their dogs (Courcier, et al. 2009).

CONCLUSIONS

Conclusions of this study are that pet owners are aware about the health risks of obesity they do not attempt for necessary diet modification and exercise due to improper perception of their pet's body condition. Therefore, current findings indicate the importance of a scientific method (based on standard criteria) in pure breed dogs to minimize obesity-related health risks.
Table 4: Attitudes and knowledge on feeding practices, necessity of exercise, obesity and scientific methods of evaluating body condition in dogs

<table>
<thead>
<tr>
<th>Question</th>
<th>N (Years)</th>
<th>N (Obesity)</th>
<th>N (Necessary)</th>
<th>N (Scientific)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q2</td>
<td>40%</td>
<td>20%</td>
<td>40%</td>
<td>20%</td>
</tr>
<tr>
<td>Q4</td>
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</tr>
<tr>
<td>Q6</td>
<td>40%</td>
<td>20%</td>
<td>40%</td>
<td>20%</td>
</tr>
<tr>
<td>N</td>
<td>69.2%</td>
<td>13.5%</td>
<td>7.7%</td>
<td>90.4%</td>
</tr>
<tr>
<td>Y</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>15.4%</td>
</tr>
<tr>
<td>DK</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

BCS (Body Condition Score):
- 1: Underweight
- 2: Normal
- 3: Overweight
- 4: Obese
- 5: Severely Obese


INTRODUCTION

Feline panleukopenia (FPL) (also known as feline distemper) is a contagious and fatal disease of both domesticated and wild felids. The disease is caused by Feline panleukopenia virus (FPLV) of the family parvoviridae. This virus is closely related to the canine parvo virus (CPV), mink enteritis virus (MEV) and raccoon parvo virus and only show <2% difference in their genome (Steinel et al., 2001; Tailor et al., 1999). In vitro culture and in vivo inoculation of virus of the family paroviridae have confirmed overlapping host ranges, and their classification is mostly based on the host from which the virus has been isolated (Shackelton et al., 2005; Truyen and Parrish, 1992). The FPLV among wild felids are less commonly reported due to difficulties in observing clinical signs, although, many evidences confirm that FPLV can affect almost all species of wild felids (Lane et al., 2016; Olmsted et al., 1992).

The Feline panleukopenia is transmitted through oronasal route from contaminated food, fomites and air. Viral multiplication in actively dividing cells (such as, bone marrow, lymphoid tissue, intestinal epithelium, cerebellum and retina of neonate kittens) cause lysis of energy and protein supplements. No oral food was permitted until vomiting ceased. She responded well to intravenous fluids (0.9% NaCl and lactated ringers) were administered to correct dehydration and electrolyte loss, and 25% dextrose and amino acids were administered as energy and protein supplements. No oral food was introduced until vomiting ceased. She responded well to treatment and recovered after 4 days.

Case 2: A three month old unvaccinated female Bengal tiger cub housed at National Zoological Gardens, Dehiwala developed haemorrhagic diarrhoea and vomiting. The faecal sample revealed eggs of Anicylostoma species, and hence she was treated with an oral anthelmintic (Drontal plus at 10 mg/ Kg BW). Fluid therapy could not be given due to difficulties in separating the cub from the dam, and the tiger cub died the following day.

Necropsy revealed a dehydrated carcass with severe hyperaemia on the serosae of the duodenum and jejenum, and petechial haemorrhages on the rest of the intestine. The dilated intestines contained haemorrhagic watery feces which tested positive for parvo viral DNA (Figure 1),
but not for Salmonella. Mesenteric lymph nodes and spleen were enlarged. Histopathology revealed shortening and collapsing of intestinal villi with extensive loss of epithelium in the intestinal crypts. In addition, (Figure 2a and 2b) marked bacterial colonization was seen in the mucosae and sub mucosae of the intestines (Figure 2b).

**DISCUSSION**

In addition to domestic and wild felids, FPLV can also affects racoon, mink and fox (Truyen et al., 2009). It is important to note that the FPLV is not the only parvo virus affecting cats, and new variants of canine parvo virus (CPV2a and b) are also capable of causing haemorrhagic
enteritis in both domestic and wild cats (Truyen et al., 1996). Passaging of FPLV and CPV2 through multiple hosts has caused mutations in the gene encoding viral capsid. Resulting changes in the capsid has broaden the host range of the virus (Truyen et al., 2009).

In order to identify the species of the parvo virus, PCR products were sequenced. The PCR assay used in the present study can screen for both CPV and FPLV. The resulting PCR amplicons were sequenced for species identification. Sequences were compared using BLASTn against the GenBank (http://www.ncbi.nlm.nih.gov/) non-redundant nucleotide collection database to identify the closest match. The results confirmed that the two sequences were identical and highly similar (96% coverage and 98% identity) to the Feline panleukopenia virus strain 42/06-G8, isolated from a cat with FPL in Italy (gene bank accession EU498699.1). Though many clinical cases with signs similar to FPL were observed among domestic cats in Sri Lanka, none of those cases were confirmed as FPLV by molecular or any other diagnostic methods. Therefore we are unable to confirm that the FPLV strain in wild felines is similar to the FPLV circulating among domestic cats in Sri Lanka.

Previous reports have shown that the FPL may develop as a clinical or sub clinical infection. Severity of the infection may depend on various factors including maternal derived antibodies, age, the immune status of the individual and underlying diseases. The concurrent worm infestation could have contributed to the severity of the infection in the Bengal tiger. Both Ancylostoma and FPLV damage the intestinal epithelium facilitating the intestinal microbes to translocate to blood and various organs causing septicaemia and endotoxaemia. In a previous study, a germ free cat had developed only mild transient infection following FPLV infections (Carlson et al., 1977), indicating that the systemic entry of intestinal bacteria determine the severity and the prognosis of FPLV infection. Furthermore, the young age of the animal (<3 months) would have contributed to the severe outcome. Previous reports have shown FPLV infected kittens to have a very high (90%) mortality (Truyen et al., 2009).

Histopathology of the tiger cub showed epithelial cell destruction mainly in the intestinal crypts. Cells in crypts of Lieberkühn have high replication rate and the FPLV initially start to grow in these cells. With the progression of the infection, villi atrophy occur due to absence of new cells to replace the old epithelium (Goddard and Leisewitz, 2010). The most common symptoms of FPL; ie., diarrhoea, vomiting and dehydration occur due to damaged absorptive surface of the intestines.

Since there is no specific treatment available for FPL infection, affected animals should be managed and treated according to the symptoms. A broad spectrum antimicrobial, antiemetic and appropriate fluid and energy corrections should be included in the treatment and management plan to avoid complications due to septicaemia, endotoxaemia or severe dehydration. It is also vital to isolate the suspected patients as FPL is highly contagious. When FPL infection is suspected in animals in captivity, extra precautions should be taken to prevent the spread of the infection. In order to prevent FPL in Zoological Gardens, all members of the cat family should be properly vaccinated. Three vaccines, at 8-9 weeks, 11-12 weeks and 16-20 weeks of age are recommended for kittens when there is a high infection pressure. A booster vaccine should be given, when animal is one year old followed by boosters at intervals of three year or more.

ACKNOWLEDGEMENT

Authors would like to thank Ayoma Hettiarachchi, K. Thanajayan, Chandima Mallawa, Ushantha Hewagamage for their support.

REFERENCES


Clinical communication

PREVALENCE, TREATMENT AND MANAGEMENT OF HEALTH CONDITIONS IN PET RABBITS PRESENTED TO THE VETERINARY TEACHING HOSPITAL (VTH), UNIVERSITY OF PERADENIYA


Veterinary Teaching Hospital, Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya

SUMMARY: Forty five pet rabbits of different breeds with various clinical conditions were presented to the VTH from January 2014 to March 2016 reflecting an emerging trend in companion animal ownership and health concerns. Of these cases, Psoroptic mange was the commonest disease condition while traumatic injuries (26.7%) including dog bite wounds, fractures, eye conditions were regularly encountered. Chemical trauma and infectious diseases (17.8%) namely, ulcerative pododermatitis, respiratory tract and urinary tract infections; miscellaneous cases such as floppy rabbit syndrome, heat stress, nutritional deficiency and prolapsed vagina were also presented. The objective of this communication is to make the veterinary practitioners aware of the common health conditions encountered in pet rabbits and how those were treated and managed.

BACKGROUND

Domestic rabbits are descended from the European rabbit, which belongs to the order Lagomorpha. Numerous breeds of domestic rabbits have been developed with various characteristics. Common rabbit breeds in Sri Lanka included New Zealand white, Flemish giant, Californian white and Chinchilla. Pet rabbits are mostly cross bred of these original breeds. Forty five pet rabbits of different breeds (pure and cross bred) with various clinical complications presented to the VTH from January 2014 to March 2016 are discussed here.

Psoroptic mange is a common mite infestation of rabbits caused by Psoroptes cuniculi. This condition was encountered in 19 out of 45 rabbits and clinical presentation of most rabbits was otitis externa with crusty exudates that form within the ear canal and extend up to the pinna and also the skin form on the nose (Figure 1), paws and the perineal region with extremely pruritic lesions. Diagnosis was confirmed by microscopic examination of the exudates; mites were also visible to the naked eye. These were treated with Ivermectin (0.4mg/kg) subcutaneously and followed-up after ten to twelve days. All the cases responded well to this treatment.

Among the cases presented 20% were traumatic injuries mostly due to dog bites and management related injuries. First case was an extensive infected laceration due to a dog bite at the base of the left ear up to the neck (Figure 2). The wound was cleaned with normal saline, dressed with Povidone Iodine solution and enrofloxacin (10mg/kg) sid SC was administered. There was evidence of healing of the wound with daily treatment but it succumbed to the condition after three days. The other rabbit aged five months was presented with a dog bite of the left hind limb, where the tibia fibula, meta tarsal and digital bones with the associated soft tissue were lost resulting in an avulsion. Patient was stabilized with intravenous fluid (0.9% saline + 10% dextrose) and analgesics (meloxicam 0.2mg/kg PO). Amputation of the left hind limb was performed under general anaesthesia (GA); induction with ketamine HCl (35mg/kg) and xylazine HCl (5mg/kg) IM. Enrofloxacin (10mg/kg) was administered to prevent bacterial infections. Even though the patient showed difficulty in hopping there was uneventful and complete recovery.

Four rabbits were presented with management handling issues causing fractures and one with epistaxis. A cross bred rabbit with closed complete right tibia-fibula fracture was treated using intra-medullary pin insertion. Pin was removed fourteen days later and the patient showed a good response. A five month old rabbit with complete closed mid tibia-fibula fracture was treated by external coaptation using plaster of paris (POP), under GA. Owners were advised to reduce movements and the POP was removed ten days later. Another three year old rabbit was reported with epistaxis after falling down from a height, and was managed using cold fomentation, application of one drop of Oxymetazoline® 3 times a day for 3 days into each nostril which resulted in complete recovery. A 6- month old rabbit presented paraplegic after fallen down while carrying by the owner. Pain sensation was present in the caudal region of the body, but no fractures were detected in the radiograph. Pain management was done using meloxicam orally (0.2mg/kg sid) for five days and the owner was advised to do physiotherapy (hot fomentation) but had a poor prognosis.

Two rabbits with hypersensitivity reaction due to skin exposure to Lysol® were presented and treated with dexamethasone (2mg/ kg) and chlorpheniramine maleate...
counts were unremarkable. Abscess at the hock was drained to be performed. A rabbit with corneal ulcer was treated with Keterolac (1 drop bid) and ciprofloxacin (10 mg/kg) and betamethasone (1 drop tid) eye drops for fourteen days which resulted in complete recovery. Ocular conditions of rabbits were also encountered. A rabbit with an eye abscess (Figure 3) was treated with enrofloxacin (10 mg/kg SC) as antibiotic therapy. It has been frequently isolated from these animals (Frances Harcourt-Brown, 2002). Information on the life cycle of the parasite is important to decide the treatment plan; eggs hatch up to 21 days (medirabbit.com, 2016; Urquhart, 1996). The antibiotic response, and the treatment protocol was similar to that of protocol proposed by Longley, 2010. Therefore, follow-ups are necessary. Owners should be advised not to remove the crust as it may cause severe pain and may also leads to wounds. This infestation can rapidly develop into serious dermatitis. Psoroptic mange can be diagnosed by observing the mite under light microscope.

Two cases with respiratory tract disease were also encountered. Nasal swab of one of the rabbits was positive for Pasteurella multocida. The antibiotic treatment was decided based on the culture and sensitivity test. If the patient responded well to the treatment, the entire group was treated. Otherwise, the group was divided into two and treated separately.

Nutritional deficiency, heat stress, traumatic injury, and ocular conditions were also encountered. Sludgy urine (0.4 mg/kg) IM after profusely cleansing off the chemical with water. Traumatic injuries due to unknown causes were also reported, such as paraplegia and scrotal laceration.

Ocular conditions of rabbits were also encountered. A rabbit with an eye abscess (Figure 3) was treated with enucleation under GA through lateral canthotomy followed by removal of the globe and tarsorrhaphy. Corneal opacity and conjunctivitis in an adult rabbit were treated with ciprofloxacin (1 drop bid) and betamethasone (1 drop tid) eye drops for fourteen days which resulted in complete recovery. A rabbit with corneal ulcer was treated with Keterolac® (1 drop bid) and ciprofloxacin (1 drop bid) eye drops for fourteen days but the response was unsatisfactory and thus enucleation had to be performed. Two cases with ulcerative pododermatitis were reported (Figure 4). They were anorexic and the lesions were manifested as ulcerated infected areas of skin/abscess on the caudal aspect of the tarsus and metatarsus. The full blood counts were unremarkable. Abscess at the hock was drained
off and a bandage was applied after applying Soframycin® ointment and in addition enrofloxacin and meloxicam were also administered SC. Owner was advised to change the bandage regularly and also to apply Soframycin® ointment and change the bedding material (hay) regularly.

One rabbit was presented with pododermatitis with concomitant psoroptic mange and was treated with ivermectin and enrofloxacin SC which resulted in good recovery.

Three rabbits were presented with respiratory tract infection. The clinical signs included loss of appetite, clear nasal discharges, shallow rapid breathing and wheezing. Treatment with Cotrimoxazole® (30mg/kg bid) and dexamethasone (1mg/kg) for five days gave a positive response, and the treatment protocol was similar to that of protocol proposed by Longley, 2010. Nasal swab of one of the rabbits was positive for Pseudomonas and Staphylococcus. The antibiotic susceptibility test indicated that the bacteria were susceptible to enrofloxacin, cotrimoxazole and ciprofloxacin.

A rabbit with a complaint of dysuria for four days, dribbling of urine, abdominal pain and reduced appetite was presented. Urinanalysis revealed high calcium carbonate crystalluria, pyuria, haematuria and pH of 9 while lateral abdominal radiograph revealed urine filled distended bladder with radio opaque sediment. The condition was diagnosed as sludgy urine and cystitis (Figure 5). Urine was removed from the bladder manually under sedation (diazepam 1 mg/ kg) IV and treated with enrofloxacin (10mg/kg SC sid), Furosemide® (1 mg/ kg IV sid), meloxicam (0.2 mg/ kg SC sid) and vitamin C (25 mg PO sid) (Carpenter, 2005) for three days. The owner was advised to supply ad libitum drinking water and change the diet to fruits and vegetables with high water content (Kestenman, 2016). The patient responded well to the treatment.

Two similar cases were presented with forward head tilt, inability to lift the head or move the fore limbs, mild dehydration and reduced appetite. Based on the clinical signs, the condition was tentatively diagnosed as floppy rabbit syndrome (Figure 6) and SC fluid therapy, enrofloxacin (10mg/kg) and dexamethasone (1mg/kg) IM were administered. Ciprofloxacin (10mg/ kg) and dexamethasone were prescribed orally for two more days. Both rabbits recovered completely.

Two cases of abscesses were present with white thick creamy content; both were present below the mandible (Figure 7). The culture was positive for Pasteurella. Abscesses were surgically drained under GA and enrofloxacin SC was given as antibiotic therapy. It has been reported that chronic suppuration is common in rabbits and slow growing, well-encapsulated, relatively painless abscesses develop readily. Pasteurella multocida has been frequently isolated from these abscesses (Frances Harcourt-Brown, 2002).

There were other cases reported such as vaginal prolapse (which succumbed to the condition few minutes after admission) reproductive failure due to malnutrition which was diagnosed based on the condition of the body. The condition of two rabbits presented with reduced appetite could not be diagnosed while another adult rabbit was diagnosed having heat stress which presented with respiratory distress, elevated rectal temperature (106°F) and sudden collapse. It recovered after giving intravenous fluid and keeping in a quiet air conditioned room.

**DISCUSSION AND CLINICAL SIGNIFICANCE**

All rabbits infested with *psoroptes cuniculi* were similar to typical psoroptic mange cases reported in literature (Frances Harcourt-Brown, 2002). Information on the life cycle of the parasite is important to decide the treatment plan; eggs hatch after four days, but the mite can survive in the environment up to 21 days (medirabbit.com, 2016; Urquhart, 1996). Therefore, follow-ups are necessary. Owners should be advised not to remove the crust as it may cause severe pain and may also leads to wounds. This infestation can rapidly spread among rabbits (Frances Harcourt-Brown, 2002). Therefore all the rabbits of the group should be closely examined when one is infested.

**Table 1: Summary of the health conditions in rabbits reported in this study**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoroptic mange</td>
<td>19/45</td>
<td>42.22</td>
</tr>
<tr>
<td>Ulcerative pododermatitis</td>
<td>2/45</td>
<td>4.44</td>
</tr>
<tr>
<td>Traumatic injuries</td>
<td>9/45</td>
<td>20.00</td>
</tr>
<tr>
<td>Ocular conditions</td>
<td>3/45</td>
<td>6.67</td>
</tr>
<tr>
<td>Abscesses</td>
<td>2/45</td>
<td>4.44</td>
</tr>
<tr>
<td>Respiratory tract infections</td>
<td>3/45</td>
<td>6.67</td>
</tr>
<tr>
<td>Floppy rabbit syndrome</td>
<td>2/45</td>
<td>4.44</td>
</tr>
<tr>
<td>Sludgy urine</td>
<td>1/45</td>
<td>2.22</td>
</tr>
<tr>
<td>Heat stress</td>
<td>1/45</td>
<td>2.22</td>
</tr>
<tr>
<td>Nutritional deficiency</td>
<td>1/45</td>
<td>2.22</td>
</tr>
<tr>
<td>Prolapsed vagina</td>
<td>1/45</td>
<td>2.22</td>
</tr>
<tr>
<td>Undiagnosed</td>
<td>2/45</td>
<td>4.44</td>
</tr>
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</table>

Ulcerative pododermatitis is a very painful and serious condition which is difficult to cure once sets in. It has been recognized as a secondary disease to physical, conformational or husbandry problem (Frances Harcourt-Brown, 2002). The most common bacteria isolated from these lesions are *Staphylococcus aureus* and *Pasteurella multocida* (Frances Harcourt-Brown, 2002).

Floppy rabbit syndrome is a condition causing generalised muscular weakness. Aetiology of this disease is not known although several possibilities exist, such as hypokalaemia, nutritional muscular dystrophy, myasthenia gravis and spinal cord disease. (Esther van Praag, medirabbit.com, 2016). A feature of this condition is that the recovery can take place with supportive care.
There is a high probability of rabbits acquiring psoriatic mange and subjected to traumatic injuries similar to the reports by Okerman, (1994). Therefore, when rearing rabbits, owners should take measures to reduce traumatic injuries such as constructing cages with a suitable floor which facilitate drainage but prevent accidental trapping of limbs; and also to provide them with proper nutrition. Mixed infection was reported only in a one case where the rabbit was having both psoriatic mange and ulcerative pododermatitis. As rabbits are animals that are prone to stress easily and thus gentle handling with minimum stress is very important in disease diagnosis and treating by the veterinarians and owners.

ACKNOWLEDGEMENT

Authors extend their appreciation to the academic and non-academic staff of the VTH for their enormous cooperation extended during this study. The dedication of the final year veterinary students of the batches 2009/2010 and 2010/2011, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya is especially acknowledged in managing the cases.

REFERENCES


THE GLOBAL PERSPECTIVE ON FOOD SAFETY AND FOOD BORNE ILLNESS

Food safety creates major public health issues worldwide. Food borne illnesses are common, costly, and a so far preventable public health problem. The food borne illnesses are defined as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food or water (WHO: Fact sheet N°237, 2007). These contaminations in food and water can create an enormous social and economic burden on a society and their health system in both developed and developing countries such as Sri Lanka. When foodborne illness is considered both biological and chemical agents are involved. The estimated reported global burden of foodborne illness is caused by 31 bacteria, viruses, parasites, toxins and chemicals (WHO, 2016). The commonly identified biological agents are Campylobacter spp., E.coli O157:H7, Listeria monocytogenes, certain serotypes of Salmonella enterica, Shigella spp, Clostridium botulinum, Staphylococcus aureus, Norovirus, Toxoplasma gondii and foodborne trematodiases (WHO: Fact sheet, 2016).

It has been reported that globally there are nearly 1.7 billion cases of diarrheal disease every year and this kills around 760 000 children under the age of five years (WHO: Fact sheet N°330, 2013). A great proportion of these cases can be attributed to contamination of food and drinking water. Developing countries such as Sri Lanka also bears the brunt of the problem due to the presence of a wide range of foodborne diseases.

PREVAILING STUDIES AND KNOWLEDGE GAP ON FOOD BORNE PATHOGENS AND ITS IMPACT

Passive surveillance studies have being carried out in Sri Lanka regarding the identification of food borne pathogens. In Sri Lanka most frequent were diarrheal diseases caused by Campylobacter spp., and Norovirus. In 2012, a total of 4003 clinically confirmed human cases of foodborne illnesses were reported. Among those, the majority were young children below 9 years of age (Annual Epidemiological Bulletin, 2012). In a study conducted in 2012 it has been found that the prevalence of Bacillus cereus was 56 % in Chinese style fried rice that found in the age group of 5-14 years old. The number of dysentery patients was 4832 cases (33.7% were children 1-4 years) and 2056 viral hepatitis cases (27.6% were 5-14 years).

Storage of boiled rice at room temperature for more than forty eight hours and the cooking frequency (Perera and Ranasinghe, 2012). Toxigenic Aspergillus species, Aflatoxin B1, Aflatoxin G1 have been also identified in parboiled rice and raw milled rice due to the increased storage time and the storage method (Bandara et al., 1991a, Bandara et al., 1991b). Ocratoxin has been identified in the consumable food samples in the North Central Province of the country (Wanigasuriya et al., 2008).

Fish collected from the Negombo area and distributed in suburbs of Colombo had been positive for the presence of Escherichia coli, Salmonella spp and Listeria monocytogenes along the different steps of the supply chain such as at the boat, the ice manufacturing plant and at post-harvest handling. Fish is important in the context of contamination as it is easily perishable (Ariyawansa et al., 2016). Salmonella species have been identified in both captured and cultured shrimp at a prevalence of 12.8 % in a study conducted in 2008. A Salmonella infection could result in nausea, vomiting and abdominal cramps (Kamalika et al., 2008). In dried fish from the markets in Kandy area the presence of different fungal isolates including Aspergillus niger and Aspergillus flavus was shown (Atapattu and Samarajeewa, 1990). Traces of heavy metals such as Ca, Cd, Cu, Fe, Hg, K, Mg, Mn, Na, P, Sr and Zn have also been found in Tilapia reared in the water reservoirs. The standards were below the international recommended levels during the year 2009 (Allinson et al., 2009).

Rajasooriya et al., (2002) have conducted a study on ground water quality at one region of Valigamam and identified a large proportion of wells having high level of nitrate which exceed the WHO standard due to intensive agriculture practices through high inputs of artificial and natural fertilizers. However, this water is used for different farming practices and also in the farmers' houses. The possible contamination of fecal coliforms in drinking water resources in Jaffna area has been documented (Muralithas et al., 2011).

Foodborne illnesses are of major concern in the YOPI (Young, Old, Pregnant and Immunosuppressed) group of people because of the reduced immunity levels (Havelaar et al., 2015, Barbuddhe et al., 2012, Kirk et al., 2015). In 2014 the annual health bulletin of Sri Lanka had indicated 1072 cases of typhoid fever were reported and 27.6 % was found in the age group of 5-14 years old. The number of dysentery patients was 4832 cases (33.7% were children 1-4 years) and 2056 viral hepatitis cases (27.6% were 5-14 years).

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http://doi.org/10.4038/slvj.v63i2.14
Some pathogens that are reported from food borne illness cases emerge as multi drug resistance type and this cause further challenge to control the disease (Garedew et al., 2015; Routh et al., 2015; Liu et al., 2013).

Continuous screening and monitoring of antimicrobial susceptibility in bacteria from any animal or plant originated food is extremely important to ensure the betterment of global public health and economy as well. The Food Microbiology Laboratory at the Department of Veterinary Public Health & Pharmacology, Faculty of Veterinary Medicine & Animal Sciences, University of Peradeniya, Sri Lanka has been participating in studies on antimicrobial resistance and residues of animal originated food, through the World Health Organization (WHO) program of External Quality assurance System (EQAS) since 2005. However, these program activities are able to produce a glimpse of what is the real situation of antimicrobial use, antimicrobial resistance, and antimicrobial and other chemical residues in water, plants and animal originated food in Sri Lanka. The WHO has also established the Food borne Disease Burden Epidemiology Reference Group (FERG) in 2010 in which Sri Lanka collaborated (Havelaar et al., 2015).

The annual health bulletin of Sri Lanka in 2014 reported the leading 11th cause of hospitalization was intestinal infectious diseases (128,733 cases) and 9th cause among the leading cause of hospital deaths. In year 2014 the ministry allocation for health was 162 billion rupees. This is mainly spent on recurrent and capital expenditure. The amount spent on recurrent expenditure was 77 % (Sri Lanka National Health account 2005-2009). The main part is spent on curative health which is for the inpatient and outpatient care. The percentage spent on inpatient/hospitalized patients was 95% from the recurrent expenditure (Public hospital governance in Sri Lanka, 2015). From this the percentage spent on gastrointestinal patient (total number of GIT infectious disease people/total number of hospitalized patients) was around 2%. This accounts for 2.37 billion rupees.

In Sri Lanka, Central Food Control administration comes under the purview of the Ministry of Health. The Food Act no 26 of 1980 enables the legal authority regarding the manufacturing, storage, sale and distribution of food in the country. This is antecedent and how well it addresses the existing need of the country is of concern. When recent past is considered there is an increasing trend towards food borne illnesses and the public interest stirrup occasionally with frequent news bulletins. The passive surveillance research already conducted in Sri Lanka has not yet been adequate to fulfill the requirement of preventing the economic loss that had been occurring in the country. Therefore more scope remains for the research activities in the area of food safety.

There are numerous successful stories where food borne illness has been successfully controlled by strengthening the surveillance system in both European and Asian countries such as Denmark, Thailand and Malaysia (INFOSAN 2014/ 2015; Pulse Net 2016).

**RISK ANALYSIS AND THE NEED OF FOOD SAFETY MONITORING AND SURVEILLANCE SYSTEM IN SRI LANKA**

The world-wide practiced approach for the identification of food borne illness is the systematic risk assessment at every step involved to rule out or rule in the suspected cause. The risk is defined as the probability of occurrence of an undesired event and its consequences. The food borne pathogen can be chemical, biological and physical in nature (Codex Alimentarius, 2010).

Sri Lanka is in need of an active surveillance system to reduce the disease burden. The country can learn from these examples and adopt according to its requirements. The world practiced approach for monitoring food safety through systematic surveillance at each step along the food chain is briefly explained.

Once a suspected case of food poisoning is reported there may be different culprits such as the environment or the food handlers but most of the time it is directed towards the food itself. In the ideal situation the samples will be examined from all the suspected routes of entry. This will be conducted via microbiological testing whether to rule in or rule out each cause. When the food hygiene is considered, it involves sequential steps from farm to table. The procedure can be categorized as pre-processing stages and post-processing stages.

If meat production is considered the preprocessing stage involves the sequential steps starting from the farm environment, animal feed, animals on farm, transport to the market or lairage, from there to abattoir and processing. The post processing stage involves storage and retail, domestic trade and retail catering to the consumers. All along the chain there are numerous routes from where the introduction and the transmission of pathogens could take place. If the chemical agents are considered it can be heavy metals, pesticides, veterinary drugs and growth promoters, preservatives and additives. The biological agents are bacteria, virus and parasites that are from water, soil and animal cross contamination.

The OIE risk definition is mainly concerned with the international product import and export. The risk is defined as “The likelihood of the occurrence and the magnitude of the consequences of an adverse event to animal or human health in the importing country during a specified time period”. Here we are concerned with the local consumption and this should be identified as the likelihood of occurrence and the magnitude of consequences of adverse event to animal/ human health during the specified time period. According to OIE, Risk assessment is “A systematic way of gathering, evaluating, and recording information leading to recommendations for a position or action in response to an identified hazard (hazard=food borne illness)”.

According to the Codex alimentarius, risk assessment is “the identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods”.
The risk assessment consists of 4 steps: (i) release assessment, (ii) exposure assessment, and (iii) consequence assessment and (iv) risk estimation.

With regard to food of animal origin at the release assessment step the veterinarian plays the key role. Release assessment is the releasing of pathogenic organism to the environment from the source. This step identifies the point where the pathogenic agent had entered the food chain both at pre-processing and the post-processing. Pre-processing stages are in the farm where apart from pathogenic organisms, the drug residues, fungal toxins and heavy metals can enter the food chain. The releasing factors can be categorized as biological, country and commodity factors. The post processing stage involves first meat inspection.

Exposure assessment expresses the biological pathways necessary for exposure of humans to the hazards (biological or chemical) released from a given risk source i.e. the frequency of contamination of a food by an agent and its level in the food over time before consumption. It should be considered although the pathogenic organism can be present at one step, at another step it can be completely destroyed by cooking at a higher temperature or freezing at a lower temperature.

Consequence assessment describes the adverse health consequences that may arise as a result of being exposed to such biological or chemical agent. As indicated earlier, causing nearly an estimated amount of Rs 2.37 billion loss and 128,733 cases per year, the foodborne illnesses trigger considerable losses to the economy and the health of the people of the country. Apart from the direct financial loss there is the associated socio-economic loss. The socio-economic impact of the diseases relates to the loss of manpower to the government and/or the private sector, and to the family of the person as a result from being sick. This is calculated by the disability adjusted life year (DALY) which is based on the loss of number of years due to the disease burden (ill health and death).

Based on the results of the risk assessment the risk estimation is done qualitatively or quantitatively. Qualitative risk assessment is carried out by developing the risk matrix where the ultimate risk is categorized as high, medium and low. Quantitative risk assessment is carried out by deterministic or stochastic models.

Once the risk is identified it should be managed to an acceptable level. This is instigated by awareness, policy making and creating standards. At international level for importation of food products it is essential to achieve Food Safety Objectives (FSO) and follow Good Hygiene Practice (GHP) /Good Manufacturing Practice (GMP).

Considering the local market, the created standard should be able to provide appropriate level of consumer protection. Special attention should be paid at the local market since Sri Lanka is becoming a popular tourist destination.

In USA, CDC has announced that by reducing the food related illness by 10%, the Americans have reduced 5 million people getting sick every year. From the consumer aspect they should be encouraged to buy the products that are certified and that can be traced back.

In conclusion the need remains for implementing a proper food safety surveillance system in the country with a multi-disciplinary approach at every step involved. With the present economic growth rate of Sri Lanka of 6.2%, the purchasing ability of general public is improving and the need for quality food products will be in demand. This will create a suitable environment for such organized systems. The government should be made aware of the role of veterinarian in this context. Further, the research capacity should be strengthened as to address how the system can be improved to meet the gap between the public health challenges and preventing the economic losses to the country.

REFERENCES


Havelaar, A.H., Kirk, M.D., Torgerson, P.R., Gibb, H.J., Hald, T., Lake, R.J., Praet, N., Bellinger, D.C., de Silva,


SUMMARY: Human-monkey conflict is often reported due to deforestation and increasing human population with their needs. This study summarises the findings of most of the work done in this regard from the year 2000 to 2016. Largely individual monkeys and/or individual lonely mothers with young ones and secondly the troops of monkeys cause conflicts within Kandy municipal area. Red faced monkeys prefer human food, home garden produce and insects found in and around houses. In addition to being a menace, these monkeys, carry some potentially zoonotic enteric pathogens. The methods launched to control this conflict were tested such as capturing, sterilizing and translocating or rehabilitating and use of repelling devices to change monkeys’ trails. The castration, and ovario hysterectomy on a portion of the troop in reducing their numbers needs debate. A completely monkey proof garbage disposal system is also a must.

INTRODUCTION

With increasing deforestation, human-monkey conflict in Sri Lanka is on the increase. The increasing human population growth and exponential increase in their needs also have contributed to the same (Nahallage, et al., 2008). In the year 2000, after several written requests reaching the Veterinary Teaching Hospital (VTH), University of Peradeniya (UOP), several programs were launched in this regard. The red faced monkeys (toque macaques/ *Macaca sinica*) is the culprit animal, making trouble to daily life of several groups of individuals, shops, schools and places of worship. This documentation summarises all efforts of VTH from 2000 to 2016 with regard to human monkey conflict.

EXTENT OF THE CONFLICT

The Veterinary Teaching Hospital (VTH) launched a project through Postgraduate Institute of Science (PGIS) in the year 2000 to study the extent of this problem within Kandy Municipal limits. It showed that there were two types of monkeys involved. Firstly, individual monkeys or individual mothers with kids. Secondly, the troops. These monkeys had been possibly tamed and later released or had been used in circuses to perform various acrobatic actions by people to make a living (Wijesinghe, et al., 2005).

These monkeys keep repeatedly coming in search of tastier human food which can be found easily and, they stay around and increase their rate of breeding. It is obvious that the monkey threat has got worse during the past 15 years and that they dislike arecanut trees and certain types of banana trees while they eat or destroy all other home garden produce (Jayalath, 2011). Monkeys eat not only plant material, but also insects that are found in and around human dwellings such as those under the roofs.

This is probably why monkeys live in the periphery of the jungles close to humans. Though, it appears that largely red faced monkeys (*Macaca sinica*) are reported to be the problem, the behavior of other species of monkeys could also be disturbing (Binduhewa et al., 2005).

Suddenly within Kandy city limits in 2012, several monkeys were found dead and some of them were found ill. Such ill and dead monkeys were brought and treated at the VTH with the help of the Police in Kandy. The postmortem performed on the dead indicated that they had possibly consumed a poison. Though this purposeful poisoning was reported in daily newspapers, nobody bothered to examine as to why it happened. The monkeys at Udawattakele, a wildlife protected area in Kandy, in 2014 were not well and feeling ill which was possibly a viral condition and a few of them even died. This appears to be an annual occurrence which settles on its own.

COPROLOGIC SURVEY AMONG RED FACED MONKEYS-ENTERIC PATHOGENS WITH ZOONOTIC POTENTIAL

In 2015, a coprologic survey (Mendis, 2016) on enteric parasites, selected bacteria and viruses with zoonotic potential in *Macaca sinica* using 105 samples from Kandy area was performed. In that study, 4 types of protozoan cysts: *Entamoeba coli*, *Entamoeba histolytica* dispar, *Giardia* spp. and *Balantium colii*, 4 types of helminth eggs: strongyle type/ *Strongyloides* sp., *Trichiurus* spp., *Enterobius* spp., *Bertiella* spp. and one bacterial species: *Shigella* sp. were found. Some parasites were found more often from monkeys from areas with close contact with humans.

CONTROL MEASURES FOR THE CONFLICT

In 2005, all trouble making individual monkeys within municipal limits in Kandy were caught using different methods; trapped and/or sedated (Wijesinghe et al., 2005). These monkeys, after capturing, were surgically castrated or ovario- hysterectomised, vaccinated against rabies and tetanus and introduced into a special cage complex. This cage complex had 8 individual cages into which the monkeys were introduced, and a common corridor. The monkeys caught as above, lived in this cage complex for 6 months. One monkey at a time, was released into the

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**HUMAN-MONKEY (Macaca sinica) CONFLICT IN SRI LANKA**

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http://doi.org/10.4038/slvj.v63i2.15
common corridor in the cage complex, made contacts with others and established hierarchy. At the end of the period, they were all in one troop together, established the hierarchy and hence were released to the wild (Rupasinghe, 2006). This troop however, was not subsequently monitored.

The medical castration of male monkeys, by injecting formalin into testicles and using human intra uterine loops on female monkeys have also been attempted with some success though the work was inconclusive (Samal et al., 2015). Issues on ethics and pain relief were some problems in that work. However, the impact of performing such techniques only on a proportion of males or/and females in a troop, on reducing their numbers is questionable.

A village in Hali-ela area launched a program to control human-monkey conflict after holding, several meetings with the villagers. All these attempts were not to kill, destroy or to develop hatred towards monkeys but to educate humans to live with the monkeys in harmony. It was pointed out in such meetings that the most feasible among the few temporary solutions was to, capture, surgically sterilize, vaccinate and to release either to the same location or to translocate. The carpenters from the village built community monkey traps. One person was employed to operate the trap (Wijesinghe et al., 2009). The people in the area were also made to understand that the welfare and the genetic make-up of these monkeys must be looked into. This program with the assistance of the Department of Wildlife Conservation subsequently became popular in other areas of the country.

Mahakanda village in Kandy conducted such a capture programme and a substantial proportions of animals from several troups were caught, surgically operated and were translocated (Jayalath, 2011). However, the monkeys from the surrounding areas infiltrated in about 6-7 months. These new monkeys though initially not destructive, became gradually so (Jayalath and Dangolla, 2011).

In 2014, the University of Peradeniya, imported 5 monkey repelling electrically operated electronic devices from India. These were fitted on pre-determined strategic points within the university premises after a detailed study takes lead attempting to solve this problem, examine the use of “hypothesis testing” lesson in the GCE A/L school syllabus in Science curriculum at that time (Rupasinghe and Dangolla, 2005).

An initial island-wide survey with several educational programs was conducted by VTH. The children in the GCE advanced levels Science stream were also used in these programs (Rupasinghe, 2006). These educational programs and subsequent investigations included teachings on potential zoonotic infections that could be transmitted to humans from monkeys. The school children learn these principals quicker than expected (Wijesinghe et al., 2003).

CONCLUSION

A survey on the international literature on the monkey human conflict, show that there is no permanent solution for this problem. Proper and responsible garbage disposal seems to be a must. In agricultural areas in Sri Lanka this recommendation may not work. None of the different approaches adopted in India, Thailand or Malaysia have shown total effectivity. Resolving this problem in cities and agricultural areas, while appreciating the living rights, welfare and genetic issues of both humans and monkeys is urgently needed. It is timely that the Kandy municipality takes lead attempting to solve this problem, examine the potential benefits and losses of the proposed solutions and make decision on mass scale introduction and adoption of a remedy to ensure decent living standard for people within municipal limits.

REFERENCES


**Letter to the Editor**

**Cattle rabies in Nawagattegama Veterinary range**

**Dear Sir,**

Rabies is an endemic disease in Sri Lanka. Most often, the disease is reported in domestic or free roaming dogs. It is rapidly progressive meningoencephalitis caused by the Rabies virus or other lyssa viruses. There are a considerable number of human deaths due to Rabies in Sri Lanka and most of these human rabies cases are linked to dog bites. Therefore, there is an enormous attention to prevent and control rabies in the dog population of Sri Lanka. However, it is important to note that the rabies in livestock can also be a source for human rabies. Due to lack of awareness of rabies in cattle, goat and sheep, exact number of cases remains undiagnosed, hence, under estimated. Here we reported five cases rabies in cattle in Nawagattegama veterinary range. Three of these cases were reported in the same farm located in Konkadawala Grama Niladari division.

One year old Jersey cattle was reported to show aggressive behaviour, drooling of saliva, bellowing and was completely off food. Clinical examination revealed that the animal has hyperthermia and nystagmus. The affected cattle was in a herd of six animals and was reared under semi intensive management system. As the condition was suspected of rabies, it was advised to keep the animal in isolation under observation. Two days later the affected animal died.

Another animal, in the same herd of the previous case, started of showing similar clinical signs and died two days later.

On 04th of April 2016, a two month old female calf in the Kahandawa GS division also died after developing clinical signs described above. Head of the dead animal was packed in ice and send to Medical Research Institute, Colombo to check for rabies virus. Laboratory results confirmed that the animal was positive for rabies.

A young cattle, aged one year and two months also developed anorexia and aggressive behaviour on 16th April 2016 and died on the following day. Head of this animal was also send to MRI to check for rabies and was confirmed rabies positive.

On 14th of May 2016, two year old cattle developed similar sign and died on the same day.

According to the farmers who owned these animals, they were not bitten by dogs. However, they were tied in nearby paddy fields during night time. It is unclear whether these cattle infected by rabies virus through a rabid dog, wild animals or through bats. Due to public health risk as well as to the economic losses to the rural farmers, it would be important to vaccinate livestock animals against rabies.

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http://doi.org/10.4038/slvj.v63i2.17
Can GnRH vaccine be used on captive elephants to postpone musth?

Dear Sir,

Four client owned, captive adult male elephants were given injections of a GnRH (gonadotropin releasing hormone vaccine; 800µg per dose). The hormone administrations were planned so that the last injection was administered approximately one month before the anticipated date of musth. Two semi wild male elephants from the Pinnawela elephant orphanage also received the vaccine. Venous blood was collected immediately before the vaccine injection for assay of testosterone.

Of the client owned elephants, one received all 3 doses and demonstrated a 3-month delay in the onset of musth together with a marked reduction in aggressiveness. Two elephants that received 2 injections showed signs of musth around the expected time, however, there was a reduction in the aggression. Conversely, one animal that received one injection came into musth, two months prior to the expected date and had a prolonged period of musth.

In the semi wild elephants, one had apparently been in continuous musth for almost 2 years when the first injection was administered and this animal came out of musth immediately after the injection and had minimal aggression. However, musth reappeared approximately 5 months after the second dose. The other semi wild elephant had a history of irregular musth twice a year but responded well to the vaccine by demonstrating delayed commencement of musth by approximately 8 months after the second injection. Testosterone levels were reduced in all four client owned elephants and a semi wild animal while the other semi wild elephant showed no changes in the testosterone levels.

These preliminary findings indicate a postponement of musth, albeit for short periods following GnRH vaccine in captive and semi wild elephants. It also appears that musth-related aggression in the male elephants is complex and possibly multifactorial and is not solely reliant on testosterone. Further studies with a large number of animals to include captive, semi wild and perhaps wild elephants will help to confirm the findings reported herein. Client compliance was a major impediment in the conduct of this study.

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http://doi.org/10.4038/slvj.v63i2.16